RENAL FUNCTION

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Second Conference on Renal Function

In contradistinction to the usual scientific meeting we like to have the presentations relatively brief and to bring out the difficulties encountered in problems rather than the neat solutions of those problems In other words we feel that the heart of these meetings is in the discussion and that only in a really informal and friendly atmosphere is it possible for people to discuss freely all of their ideas Therefore we hope that you won t hesitate to speak spontaneously and informally What you say may not be too wise but how can you be sure that it won t evoke wisdom in someone else!

One point which should be *stressed* is that between the disciplines there are real difficulties in communication — partly emotional and partly semantic Emotionally some investigators accept only data derived from methods or disciplines with which they are familiar On the semantic level the physical and biological sciences can understand each other without difficulty as can the medical psychiatric and social sciences However to bridge the gap between the physical and biological sciences on the *one* hand and the psychological and social sciences *on* the other is very difficult Through the Conference Program and in the published transactions we hope to give a clearer reproduction of what takes place in the laboratory and what goes on in the minds of scientists than now appears in the scientific literature Unfortunately somewhere along the line scientists themselves have been responsible for hiding the artistic creative aspects of scientific endeavor behind the cold, white light of logic the essential testing method which makes possible the practical application and use of the creative idea

This program is an experiment and you are part of that experiment The success of the undertaking is measured entirely by what each participant gains from this experience We hope that here you will feel the freedom inherent in the scientific method and that you will help us to improve our conference procedure

INTRODUCTORY REMARKS

ROBERT F PITTS,
Chairman

It is my pleasure to welcome you as members and guests of this Second Conference on Renal Function sponsored by the Josiah Macy Foundation, and to wish you a very pleasant and profitable series of discussion sessions today and tomorrow

The environment which has been prepared by Dr Fremont-Smith, Miss Freed and the Foundation staff is a most favorable one Furthermore, this assembled company has as one of its many assets congeniality I am, therefore, sure that we will have a pleasant time I would also like to emphasize the statement of Dr Fremont-Smith that it will be a profitable time only if we freely and openly discuss our problems, contribute our own experience and seek the council of the assembled group

The topic chosen by you at the last session, namely, hormonal control of renal function, has been narrowed by me to include only pituitary and adrenal cortical influences The latter, especially, is a timely topic for discussion, since the adrenals are now so much in the public eye However, we all realize that, important as they are, the adrenal cortical hormones operate through a background of other hormonal activities, perhaps through a background of nervous vasomotor regulation, and certainly through an inherent or autonomous capacity of the kidney to work independently Nevertheless, some have questioned the wisdom of diffusing our efforts by considering two endocrine systems rather than concentrating on one, obviously the adrenals, in an effort to exhaust them in five straight sessions My answer is that our adrenal reserve is very great and that we cannot exhaust it in one conference Furthermore I feel that maintaining a proper perspective is more important than exhausting a single phase of hormonal regulation That perhaps is adequate justification of the admixture of anterior pituitary, posterior pituitary, and adrenal cortical influences which you observe in the conference program At least it is my justification We shall start our program with a consideration of the action of the adrenal cortex and related hormones on water excretion I have asked Dr Gaunt, Professor of Zoology at the State University of New York Medical Center at Syracuse to open this discussion

ADRENAL CORTICAL HORMONE AND RELATED HORMONES IN WATER METABOLISM*

ROBERT GAUNT

Department of Zoology, College of Medicine
State University of New York Medical Center at Syracuse

I WANT to begin with a little matter of personal orientation that to me at least is pertinent I was very pleased when Dr Pitts invited me a year ago to attend the first of these conferences on renal function but also embarrassed because I felt I had nothing to contribute to the subject Although we have studied various aspects of water metabolism for some time, any precise work on kidney function itself has been of a most elementary sort. There is however, one personally reassuring feature — today we talk about the adrenal and the kidney and when it comes to the adrenal I have no such inhibitions as those mentioned for the kidney Indeed if adrenal workers had kept still when they did not know what they were talking about the history of physiology during the last quarter century would have been very drab — a fact that encourages me proceed

When we discuss the effects of cortical hormones on water excretion we are faced immediately with a dilemma, for these hormones may either inhibit or they may enhance the excretion of water by the kidney I have been interested to note that among my friends only casually interested in this subject, some regard the cortical hormones as diuretic agents some as water retaining agents

The same is true of sodium excretion Although the adrenal hormones are best known as agents which cause sodium retention, there are numerous circumstances and conditions in which they enhance sodium excretion Dr Pitts and his colleagues(1) have shown that the renal tubule of the adrenalectomized animal is not always deficient in its ability to reabsorb sodium it is only deficient when the plasma sodium level is low At high plasma sodium levels the renal tubule of the adrenalectomized dog reabsorbs more sodium than normal from tubular urine

* The work referred to here from our laboratory was conducted primarily by Drs J H Birnie W R Boss W J Eversole and C M Osborn It was aided by grants from the U S Public Health Service and Ciba Pharmaceutical Products Inc

So in the midst of this rather bewildering variability of response to cortical hormones or to their absence, about the only generalization that can be made is that no generalization is possible. We are dealing with hormones of adaptation and they do different things under different circumstances.

We could bring to bear on this problem a great mass of facts and figures from many sources which would take a long time to present.* I would prefer to let them come out in the discussion, and in order to get the discussion going limit myself to some broad statements and suggestions as to the pattern of endocrine interrelationships and interactions which impinge upon the kidney and affect water metabolism. I will take advantage of the fact that in these sessions we have been invited to indulge in speculative considerations and in discussion of work in progress.

Now one of the clearest and best agreed upon facts with which we might begin is that an adrenalectomized animal cannot excrete a water load rapidly. Why is this the case? There are a combination of actual or possible reasons.

A) There are extra renal factors — one being a delayed intestinal absorption of fluid(8). Although with large water loads that factor may be of considerable magnitude it is of relatively little importance to the question here, because it is easy enough to show that if the water is absorbed or if one by-passes the gut in administering it, it still cannot be excreted at a normal rate.

B) After adrenalectomy, there may be — although not invariably — a reduced renal plasma flow and glomerular filtration rate (GFR) (4,9). The filtration rate in certain circumstances may be severely depressed — in which case presumably it could contribute markedly to a delay in water excretion. It is easy enough to maintain the GFR in adrenalectomized animals, as many workers have done, but water still cannot be excreted normally.

C) There is an enhanced tubular reabsorption of water(1,4,9, 10,11,12). I believe that has been seen by all workers who have looked specifically for it and it is probably the major cause of the inability to excrete water after adrenalectomy. This abnormality is very difficult to repair. Conversely, it is easy to show that the rate of water diuresis in normal animals and men can be greatly

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* The subject matter of this discussion has been extensively covered in recent reviews(2,3,4,5,6,7). The references cited here therefore will be largely to these reviews and to very recent papers.

exceeded when water loaded animals are given extra amounts of cortical hormone(4) This effect occurs without essential elevation in the filtration rate and hence is due to an inhibited tubular reabsorption of water

While these effects of cortical hormones on water excretion are presumably not entirely independent of sodium excretion they cannot be in many instances correlated with sodium excretion in any way to suggest casual relationships(4 13 14 15)

What then is the cause of this enhanced tubular reabsorption of water after adrenalectomy — a defect of such magnitude that an animal may be thrown into a state of lethal water intoxication at relatively small water loads? For various reasons we have been favorably disposed to the idea that it is due to a hypersensitivity to or an accumulation of antidiuretic humoral agents perhaps of posterior pituitary origin

This brings us to the concept of a functional antagonism between the hormones of the posterior pituitary and adrenal cortex — a concept that was first elaborated by Silvette Britton and Corey (16) some ten years ago *This hypothesis in its simplest form states that* the cortical hormones enhance water excretion and inhibit sodium excretion and that antidiuretic hormone of the posterior pituitary (ADH) has precisely opposite actions There are many of us who would now accept this as a general rule with certain definite exceptions It might be mentioned that both cortical and posterior pituitary hormones enhance the excretion of potassium probably acting in a supplementary rather than an antagonistic fashion(17)

I think there is little doubt that a hypersensitivity to ADH can be demonstrated in the adrenalectomized animal(18 19) but such animals are probably hypersensitive to any antidiuretic influence Various workers including our group have found evidence of the accumulation of some antidiuretic substance in the body fluids of adrenalectomized animals(20 19 21) Pitts and his co workers(1) have elaborated the idea that the increased sodium excretion of adrenal insufficiency is due to an action of ADH unantagonized by cortical hormones Therefore it may be suggested that the unantagonized action of ADH or some similar antidiuretic agent is at least one cause of the failure to excrete water and to retain sodium in adrenal insufficiency

If this be acceptable at least as a working hypothesis the question arises as to the identity of the antidiuretic substance that accumulates or is hyperactive after adrenalectomy

In the first place none of us have conclusive proof that the substance which accumulates in the body fluids of adrenalectomized animals or Addisonian patients is actually ADH It could be ADH, it could be a substance which mimics ADH, it could be a substance which causes the release of ADH Nevertheless, there is something there which disappears from the blood of hypophysectomized rats and which has the attributes of ADH(19) I will not elaborate upon these points because they will probably be discussed by Dr van Dyke

There are reasons why it might be expected that ADH would accumulate in the absence of the adrenals It has been shown that various tissues, particularly hepatic tissue inactivate pitressin(22, 23) Birnie's work(21) in our laboratory has indicated that this hepatic agent is an enzyme (probably not a specific one) and that its ability to inactivate pitressin is reduced after adrenalectomy

van Dyke May I ask whether the differences shown in your hepatic inactivation experiments with pitressin have been examined for their statistical significance?

Gaunt Yes Dr Birnie did The significance of that kind of observation will not be fully apparent until it is related quantitatively to what goes on in the whole animal Nevertheless it obviously provides a basis for the suggestion that ADH may accumulate after adrenalectomy because it is not destroyed at a normal rate On theoretical grounds one would not expect hypersecretion of ADH after adrenalectomy

I think work of this sort may have widespread usefulness in related studies Dr C H Lloyd at Syracuse has some evidence* that the liver of cirrhotic patients cannot inactivate pitressin normally either *in vivo* or *in vitro* That obviously would correlate with the work of Ralli Hoagland and their associates(25 26) concerning the genesis of the abnormal fluid metabolism in liver disease

Dr E Moller Christiensen of Copenhagen told me in a recent letter that he has shown that in water intoxication in rabbits the liver loses its ability to inactivate pitressin Such an observation might be related to the fact that as animals go into water intoxication there is at a certain point a sharp break in the diuresis Admittedly the posterior pituitary should not be active at all under these conditions however the conditions are extreme ones characterized by profound neurological and metabolic abnormalities Usual rules may not be followed

* Unpublished data

My colleague Dr J H Birnie who is working in Dr Hans Heller s laboratory in England this year tells me they have found that the livers of protein deficient animals inactivate pitressin less effectively than normal Obviously again this may be associated with the water retention seen in such animals

As far as what happens in adrenal insufficiency is concerned I want to emphasize the point that the general interpretation which has been offered to explain the aberrations of sodium and water metabolism in adrenal insufficiency is not dependent upon an accumulation of ADH It would buttress the case if this were true but if there is some ADH present in effective amounts after adrena lectomy a hypersensitivity to it might explain the tendency tc excrete water slowly and to lose sodium and chloride You ma recall in this connection that Winter Ingram and Gross(27) showed that the fall of plasma sodium in the adrenalectomized cat was dependent upon a functional posterior pituitary

The most common problem in the clinical use of DCA or cortisone is a retention of water that may lead to frank edema And this brings us back to our original statement that the cortical steroids may be either diuretic or water retaining agents

We have tried to reduce to diagrammatic terms the work and thinking(4 28 29 30 31) of many people relating to this apparent paradox (See Figure 1)

As indicated we think that the major diuretic action of steroids such as DCA is brought about by their inhibition of the tubular reabsorption of water Most people would agree that the major water retaining action of DCA results from its stimulation of salt reabsorption Under some conditions as with a large water load the diuretic factors seem to be dominant Under other conditior which are yet to be precisely defined the water retaining factors are dominant If however one gives the hormone for some time the resulting retention of salt may lead to thirst polydipsia and polyuria In the end a combination of factors would contribute both to a large water exchange and an expanded extracellular fluid volume i e to retention of fluid Such a combination of events is a probable explanation of the DCA induced diabetes insipidus like syndrome observed by the group at Columbia(29) To see the operation of this syndrome in exaggerated form it is only necessary to combine DCA administration with a high salt intake

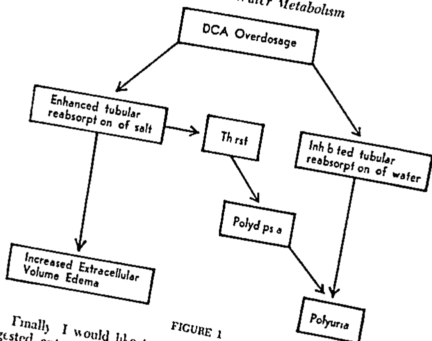


FIGURE 1

Finally I would like to explore a little the nature of this suggested antagonistic interrelationship between the adrenal cortex and posterior pituitary Certainly the hormones of these two glands do have some antagonistic properties The question is are those hormones secreted and do they act in a closely integrated check and balance system or does each follow its own more or less independent way with the antagonism being incidental? Dr Shannon raised that question several years ago(32)

If they operate in a check and balance manner it might be expected that the administration of a slight excess of one of these hormones would call forth a compensatory hypersecretion of the other

We have obtained suggestive but not conclusive evidence that a large excess of cortical hormone increases the antidiuretic activity of blood serum(19) Skahen and Green(33) found that DCA caused the excretion of an antidiuretic substance in urine These experiments were all designed for other purposes and more work is needed particularly with the use of low doses before their applicability is clear to the question at issue here

Ralli Are you speaking of DCA specifically or of other hormones

Gaunt Skahen and Green(33) used DCA We used both extract and DCA and found increased antidiuretic activity of serum but at the time we were looking for something else and we do not have enough data to establish the significance of the results

More precise quantitative information has been obtained by the opposite approach to the problem in work conducted by Dr Nagareda(34) in our laboratory She tried to determine whether an excess of pitressin would cause a compensatory activation of the adrenal cortex She had the advantage of a sensitive method, namely, the Sayers ascorbic acid depletion test for adrenal stimulation She found, in brief, that large doses of pitressin (100 or 400 mU) would cause marked adrenal stimulation as judged by the ascorbic acid depletion test This may have been due to a nonspecific stress like reaction In any case small but physiologically effective doses (5 mU) of pitressin produced no evidence of adrenal stimulation This leaves us with no convincing reason for saying that slight variations in the circulating levels of either ADH or cortical hormone *per se* causes compensatory hypersecretion of the other

There is however, another approach to the problem It is in accord with all current theory that excesses of water inhibit the secretion of ADH and thus allow such excess water to be excreted rapidly Rapid water excretion cannot occur, however, in the absence of some effective amount of cortical hormone When excess water is present and ADH secretion is more or less halted, is an increased amount of cortical hormone released to help as it were, push the excess water out? Dr Nagareda has investigated this by finding what water load would cause a measurable depletion of adrenal ascorbic acid and therefore, presumably, adrenal stimulation She found that whereas a small water load (5 ml in a 220 gm rat) would not cause adrenal stimulation a larger amount (10 ml in a 220 gm rat) would do so Thirty milliliters of water, given in three doses, was still more effective We concluded from this that whereas small water loads presumably are handled simply by reducing the output of ADH, heavier loads elicit adrenal stimulation which contributes to the diuretic process

When excess water is present, it is generally believed that the stimulus which inhibits the posterior pituitary is a fall in plasma osmotic pressure What is the stimulus to which the adrenal cortex responds when it is stimulated to activity by a water load? (What ever it is, it might be expected to be one that acts on the anterior pituitary and thus indirectly on the adrenal cortex Whether that

is actually the case is being currently investigated.) We do not know what the stimulus is but are designing further experiments on the basis of a hint emerging from this data that the adrenal might be responding in a converse manner to the same stimulus that is acting on the posterior pituitary.

All of these water doses would be expected to reduce the plasma sodium level and we have verified that they do so. If one gave normal saline instead of water there was no fall in adrenal ascorbic acid even when the doses of saline were huge (30 ml.). Can it be then that the basis for such integration as exists between the adrenal cortex and posterior pituitary lies in the fact that their hormones are "turned on" or "turned off" in a converse manner by the same stimulus, namely, the osmotic pressure of plasma? We have not answered the question — there are other alternatives — but it will be an interesting one to investigate further. Or can it be that one reason a load of normal saline is excreted less rapidly than a load of pure water is that the water may evoke an adrenal participation of a quantitative or qualitative sort which saline does not? I raise that question also without attempting an answer, but it too is one susceptible to direct experimental inquiry by methods now available.

In summary, there are several known means by which the hormones of the adrenal cortex can affect water metabolism: a) by inhibiting the tubular reabsorption of water; b) by stimulating or inhibiting the tubular reabsorption of sodium and other electrolytes; c) by maintaining the filtration rate; d) by maintaining an enzyme system in hepatic and perhaps other tissues which inactivates antidiuretic hormone; e) by maintaining a normal rate of fluid absorption from the gut; and f) by preventing an accumulation of antidiuretic substance (ADH?) in the blood. The sum total of these effects is generally to produce an enhanced water diuresis. In certain conditions, however, cortical hormone induced salt retention may lead to a fluid retention and edema or indirectly to a diabetes insipidus-like syndrome. The actions of cortical hormones on water excretion and to a certain extent those on electrolyte excretion are antagonized by the hormones of the posterior pituitary.

DISCUSSION

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ments one must always evaluate whether in attempting to obtain a definite response one has gone beyond the range of physiological dosage into what might more properly be termed a toxic dose. The fourth point is that in considering the relationships of adrenal steroids to antidiuresis it is well to recall that in man desoxycorticosterone acetate in quantities sufficient to establish normal electrolyte balance does not correct the abnormal water tolerance test (Robinson, Kepler, Power), whereas 11,17-oxysteroids do in a high proportion of patients.

Gaunt: I don't like to see you rule adrenal cortical extract out of the picture.

Thorn: I do not feel that adrenal cortical extract studies should be excluded from consideration. I do feel, however, that it is extremely difficult to interpret a negative or a positive finding when an adrenal extract of unknown composition is employed.

Gaunt: As yet we have not been able to stimulate diuresis markedly with cortisone in normal animals. We can with whole extract. There may be something qualitatively different or it may be due to a slow absorption of cortisone in our short term experiments. Cortisone will work in adrenalectomized rats.

Thorn: In man we have been able to get a marked effect. In patients with Addison's disease we have definitely shown that cortisone inhibits the increased antidiuretic activity present in these patients. A demonstrable effect may be observed with 100 mg. of cortisone given for a few days. However, in these patients it is necessary to treat them for a month or two with 25 mg. daily to obtain maximum response. The difference between the effects of desoxycorticosterone-like steroids and cortisone-like steroids emphasizes again the difficulty of interpreting positive or negative results with extracts of unknown composition.

Gaunt: That is true.

White: Did I understand correctly that the diuretic response to a 10 ml. dose of water is greater with adrenal cortical hormone administration than without? Or is it merely that these hormones protect against the water intoxication produced by excessive doses of water?

Gaunt: With cortical extracts you can get it with small water loads.

White: In acute experiments?

cortical hormone would produce a greater diuresis than with the water alone

Gaunt Much greater

Fremont-Smith What, roughly speaking, is the maximum amount that you can get with water alone? A 100 percent increase?

Gaunt It is closer to 50 or 75 percent

Fremont-Smith That is without an increase in glomerular rate?

Gaunt In water intoxication it has not been measured There is no rise when diuresis is increased following smaller water loads(9) It is almost impossible to produce water intoxication when cortical extract is given with the water We have given up to twenty of our standard doses at half-hour intervals — at the end of which time the investigator is worn out and the rat is still going strong

Fremont-Smith Was there any major difference in the composition of that urine than if they were getting straight water?

Gaunt As far as sodium chloride is concerned it was much the same but the experiments were complicated by the fact that the animal going into water intoxication develops severe diarrhea We had no means of separating completely the intestinal and renal electrolytes The total overall loss was about the same in treated and control animals Salt itself, of course, will prevent water intoxication The cortical hormones do not give protection as a result of salt conservation

Thorn There are four general comments which might be appropriate at this time First, it is important to bear in mind the fact that the *zona glomerulosa* of the rat has been shown to persist after hypophysectomy and that in rats, therefore this area of the adrenal cortex may not be under control of ACTH It is assumed generally that the zona glomerulosa secretes the desoxycorticosterone like steroids In contrast, in man it appears that all known functions of the adrenal cortex are stimulated by hog beef, and sheep ACTH preparations The second point which must be borne in mind in attempting to interpret experiments carried out with whole extract is that in most instances the exact content of steroids in such extracts is not known The third point to consider is the general principle that whereas one obtains a certain effect with small doses of steroid hormones, there may be a complete reversal of this effect with very large quantities of hormones Therefore, in evaluating animal experi-

Shorr I wonder whether that is necessarily the case with respect to adrenal cortical extract? For example to maintain rats we have found it necessary to give as much as 1.5 ml a day of adrenal cortical extract which would seem a disproportionately large dose on any human weight basis. In other words it is very hard to transfer to the rat the dosage requirements that we would assume for other species.

Thorn With adrenal extracts one must consider the rapid absorption as a factor in diminishing the efficacy of a given dose. It has been shown that rapid small injections of extract are the most efficient means of administering hormone of this type. The maintenance dose for patients with Addison's disease under normal circumstances is small whereas very large quantities of aqueous extract are necessary to reverse the water test or the abnormal electroencephalographic changes.

Ralli Dr Pitts mentioned in his opening remarks that the adrenal hormones are operating on a background of other cellular activities. I believe this to be an important consideration. The nutritional situation within the cell may affect the reaction to a given dose of a given hormone. There are substances particularly some of the fractions of the vitamin B complex which are involved in many of the coenzyme systems within the cells. It may well be that the state of these enzyme systems will determine the activity of a hormone. We have observed that the amount of pantothenic acid in the diet will affect the response of the white blood cells in intact and adrenalectomized rats to stress and to ACTH(35).

Thorn May I ask Dr Pitts if Dr Gaunt quoted him correctly when he said that he believes that the serum sodium level is the governing factor relating to sodium excretion in the absence of the adrenal?

Gaunt What I meant to say was that at low plasma levels the adrenalectomized animal reabsorbed less sodium than normal and that at high plasma levels it reabsorbed more than normal.

Pitts That is the concept we had.

Burnett However as I will bring out tomorrow the ability in umans with Addison's disease to excrete an excess load of hypernic saline is not precisely related to the serum sodium level ibular reabsorption of sodium to be sure may be as demonstrated Dr Pitts increased only at a high serum sodium level(1) but

Gaunt Yes

White Administration of the cortical hormone simultaneously with the water causes the water to be excreted faster?

Gaunt Yes With small water loads one sometimes sees it with DCA and sometimes not We have never failed to see it with extract

White Are large doses of DCA required to increase rate of water excretion?

Gaunt That is a relative matter again

White Does it take a milligram or less than a milligram?

Gaunt The dosages we have given have been generally more than that We don t know the minimal effective dose

White More than a milligram is a rather large dose for a rat

Gaunt I used to think so In terms of what the adrenal cortex itself can do I don t know what a big dose is any more

White Compared to what is necessary?

Gaunt You cannot go by that Certainly what it takes just to maintain life say that slight residual amount which the adrenal of the hypophysectomized animal can put out bears no quantitative relation to what the normal adrenal does anyway I think the work with whole extract is more meaningful Here 2 ml or so of the Upjohn product will give a good response and that is not an excessive amount by any criterion

Thorn It is important to consider the dosage employed in these experiments In an adrenalectomized animal each increment of adrenal hormone administered may be considered to be an actual increase in effective hormonal dose whereas when adrenal preparations are administered to animals with an intact adrenal it is well known that an inhibition of pituitary ACTH production by the administered hormone will result in subsequent atrophy of the adrenal cortex Under these circumstances the net effect of any given quantity of adrenal hormone administered will have to be calculated as the difference between the initial secretory activity of the gland and the total dosage given It is obvious that at a certain dosage level the administered hormone will do no more than replace endogenous hormone This is most likely to be a serious factor in considering the results of the experiments when the adrenal is already at a high level of activity before substitution of hormone

Luetscher Cortisone or ACTH In Figure 2, let us confine our attention to the bottom two curves, representing urine volume and sodium and to the weight curve, which is the continuous line at the top The patient, a five year old girl with nephrosis, was being treated with cortisone During the initial stage, there was a gain in weight which was apparently related to a diminution both in urine flow and in the excretion of sodium

In contrast to that, Figure 3 shows the course of a patient whose urine sodium was virtually zero right from the start and in whom the action of cortisone apparently was not mediated through the retention of sodium At the same time, however, the urine volume fell and a gain in weight occurred The serum sodium concentration fell during this stage Then after the fifth day there was a tendency

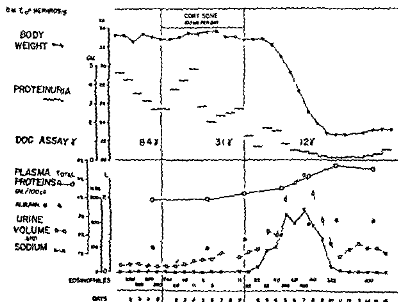


FIGURE 3

Prolonged clinical *remission following* administration of cortisone

Note temporary rise in *proteinuria* and weight during therapy with some improvement *after* the fourth day of therapy *followed* after end of treatment by elimination of over 10 kg of *edema reduction of* proteinuria to less than 0 5 *gm per day* and an increase in serum *protein* and albumin concentration approaching *normal levels Assay of* sodium retaining corticoids fell *to normal* levels after treatment Clinical *improvement* has been maintained *for* four months

the ability to excrete a load of hypertonic saline was impaired in our human experiments at both low and high serum sodium levels

Luetscher May I ask Dr Gaunt one question about interpretation? We have all observed patients under treatment with cortisone or ACTH whose body weight has first increased for a few days and then subsequently has decreased again Most of us have interpreted this diphasic response as Dr Gaunt's scheme indicated that the initial retention of water was related to the retention of sodium and that subsequent loss of water might be due to some opposite phase of action However, in the nephrotics we have run into a situation where the patients show a similar retention of water and a gain of weight during the first few days of treatment without any change in the excretion of sodium

Thorn This is cortisone, not ACTH?

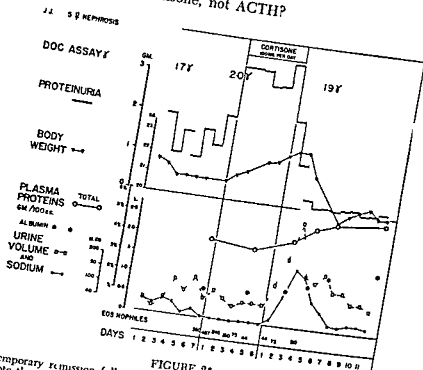


FIGURE 2*

Temporary remission following administration of cortisone

Note the increased proteinuria diminished sodium excretion and rise in weight during administration followed after therapy by cessation of proteinuria diuresis and return of serum protein and albumin concentration to virtually normal levels Edema recurred two weeks later

* Figures 2 and 3 are reprinted from a paper by J A Luetscher Jr and Q B Deming Treatment of nephrosis with cortisone *J Clin Investigation* (in press)

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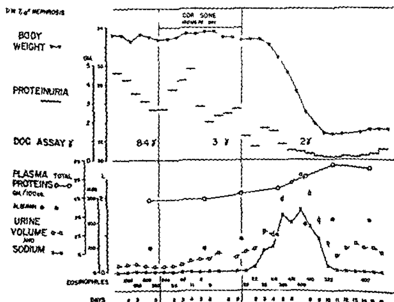


FIGURE 3

Prolonged clinical remission following administration of cortisone.

for the body weight to decrease again, as the urine volume increased, again with very small changes in sodium excretion During this stage, the serum sodium concentration increased.

In answer to Dr Thorn's question about ACTH, Figure 4 shows the course of a patient treated first with cortisone, and later with ACTH Both with cortisone and ACTH, we see a tendency for the weight to increase during the initial stage of treatment and to fall during the later stage of treatment with the expected changes in urine volume Yet the urine sodium at all these stages is insignificant until the onset of the final diuresis at the end of ACTH administration The fall in serum sodium concentration during the initial water retention, and the rise during the phase of diuresis are evident both with cortisone and with ACTH These findings suggest that cortisone and ACTH may have some form of antidiuretic activity not mediated through the retention of sodium alone

Thorn Would you say that there is a rough clinical parallelism between the degree of edema and the effectiveness of a given

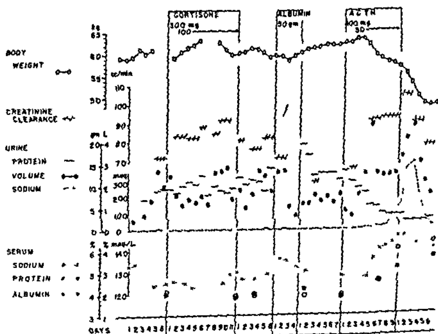


FIGURE 4

Some measurements on a patient treated successively with cortisone albumin and ACTH

quantity of these agents? The greater the edema the more likely one is to obtain a good diuresis whereas the closer the patient's weight approaches normal the more difficult it is to induce a good diuresis with either cortisone or ACTH?

Luetscher That seems to be true

Thorn It is not always?

Luetscher I was speaking primarily of the changes which were seen during the early days of treatment and which seemed to indicate some water retaining activity of cortisone and ACTH It is interesting to observe at the same time that in the patient described the creatinine was increased during this phase when the water was being retained and it seems very difficult to interpret except as a primary tubular action The changes which occur during the final diuresis are complex involving filtration rate tubular function and extra renal factors

Thorn Since patients on a very low sodium intake may retain only 5 or 6 mEq of sodium a day and show no additional excretion of potassium before diuresis occurs it is difficult to assign an important role to sodium retention and potassium loss as factors conditioning the response to ACTH and cortisone The diuresis which occurs on cortisone or ACTH with evidence of adrenal cortical activation would appear to be on a different basis than a diuresis initiated after ACTH or cortisone has been discontinued when temporary adrenal cortical insufficiency may be a facilitating factor in the sodium loss

In 1938 we reported that 11 17 oxysteroids induced increased sodium excretion when administered to normal dogs and to adrenalectomized dogs maintained on desoxycorticosterone(36) When adequate quantities of cortisone first became available for clinical trial we observed that 100 mg of cortisone daily induced sodium chloride retention These patients were not receiving desoxycorticosterone acetate The paradox appeared to be resolved when patients with Addison's disease were given desoxycorticosterone acetate in 5 mg doses per day followed by the addition of 100 mg of cortisone acetate Under these circumstances we did not observe an additive sodium retaining effect but rather an increase in sodium excretion above the base line excretion observed on desoxycorticosterone Our impression at present is that all three varieties of adrenal steroids 11 desoxy 11 oxy and 11 17 hydroxysteroids are salt retaining compounds the last being relatively inactive in

this respect There does appear to be the possibility that under certain conditions 11 17 hydroxysteroid compounds compete in action with 11 desoxy compounds under which circumstances less sodium retention will occur In our experience it is not possible to maintain patients with Addison s disease on 25 mg of cortisone daily in a normal salt balance without the addition of large quantities of sodium chloride or a small maintenance dose of desoxycorticosterone acetate whereas this quantity of cortisone will usually result in restoration of the electroencephalogram toward normal and reversal of the abnormal water test It is true that adequate salt retention may be obtained in patients with Addison s disease given 100 mg of cortisone daily but this dose is excessive and results in hypercorticism The majority of our patients have done extremely well over long periods of time on a dose of cortisone of 12 5 to 25 mg daily with 1 to 2 mg of desoxycorticosterone daily During periods of stress much larger quantities of cortisone are needed i e 100 mg daily

In patients whom we have studied with complete bilateral adrenalectomy for hypertension it is noteworthy that a normal sodium chloride balance cannot be maintained with 25 to 50 mg of cortisone daily and that ultimately supplementary sodium chloride or small doses of desoxycorticosterone are required These studies raise three interesting points First it would appear that 11 17 hydroxysteroids are not the only hormonal secretions of the adrenal cortex since they are incapable of inducing sufficient sodium retention in most patients with Addison s disease and in bilaterally adrenalectomized hypertensive subjects when given in a quantity adequate to reverse the other known disturbances This fact suggests that other substances secreted by the adrenal with greater salt retaining capacity are essential for normal function These could be either 11 desoxy or 11 oxy compounds such as compounds A and B Second in the treatment of patients with adrenal insufficiency there appears to be an optimum ratio between the quantities of cortisone and desoxycorticosterone which are employed requiring approximately 5 to 10 mg of cortisone to 1 mg of desoxycorticosterone Third in relation to Dr Gaunt s experiments it may interest you to know that in comparison to cortisone the adrenal extracts such as Eschatin of Parke Davis and Co and Upjohn s Adrenal Cortex Extract are salt retaining indicating the presence of substances other than compounds E or F

Burnett As I understand it your finding is that average doses of DCA plus small doses of cortisone cause salt loss. What about the average dose of DCA plus large doses of cortisone? We found no such salt loss with 100 to 200 mg of cortisone a day in Addisonians on maintenance quantities of DCA

Thorn I believe that the sodium chloride response of a patient with Addison's disease will be modified significantly by the dosages of desoxycorticosterone and cortisone employed. If the dosage of desoxycorticosterone is small and the dosage of cortisone very large one may obtain an overall change in the degree of salt retention

Another important point is that with cortisone acetate given intra uscularly all these effects observed in man or animals are slow fects. If you want an immediate renal effect cortisone is best en intravenously. Dr Gaunt's point regarding the aqueous extracts is a very good one because these are very rapidly absorbed. ACTH effect is observed within three to four hours after administration whereas an intramuscular injection of cortisone acetate does not attain maximum action for three or four days on a constant dose level. Recently we have shown that very rapid absorption and action as far as the eosinopenia is concerned may be obtained with cortisone administered orally

Gilman How do you give adrenocortical steroids intravenously?

Thorn We have given cortisone intravenously slowly as a suspension in saline. As with DCA we have used the glucoside intravenously

Luetscher When you speak of additive or competitive effects against the salt retaining hormone I wonder whether we ought to try to distinguish between the additive and competitive effects of cortisone versus desoxycorticosterone as the crystalline compound and versus the normal secretion of the adrenal

Have you seen any evidence of sodium loss during cortisone treatment in normal patients aside from the secondary release of sodium and water retained during the initial days of treatment?

Thorn I would think Dr Luetscher is right. Of course in all of our normal studies we have gone to high dosage levels to be sure that we are giving an excess. However I would say that in the majority of experiments 100 mg of cortisone almost invariably causes some salt retention initially in a normal individual

ing severe hypoglycemic manifestations, b) abnormal electroencephalogram (36,38) and electrocardiogram, and, c) excessive antidiuretic activity with a positive Robinson-Kepler-Power *water test* Cortisone, in adequate doses, will in almost all *instances* correct these metabolic defects The effect of cortisone may be limited in the presence of hypothyroidism and obviously cortisone therapy in maintenance doses will not restore insulin sensitivity since in a large measure this is dependent upon the intact adrenal medulla as well as upon intact liver glycogen stores

White That is what I really wanted to ask How does the addition of cortisone to DCA regimen change the salt balance? If the patient was in salt balance on DCA alone does adding 5 mg of cortisone per gram of DCA change that salt balance?

Thorn Definitely, and we have seen patients admitted in near-crisis when they were given 12 5 mg of cortisone, although on the same dose of DCA which had been adequate in the preceding year

Burnett If the cortisone is increased is the process reversed?

Thorn If you go to the larger dose With 100 mg of cortisone, I would assume that such a patient might remain in adequate salt balance but would show signs of hypercorticism We have no patients carried for a long period

Gilman Do you have any idea what metabolic defect you are correcting with cortisone when you are changing the abnormal electroencephalogram to normal? Is it related to water metabolism or carbohydrate metabolism?

Thorn We have no idea as to the mechanism of cortisone in correcting the abnormal electroencephalogram

van Dyke What are the electroencephalographic changes Dr Thorn?

Thorn We are using the changes in the *electroencephalogram* as evidence for a metabolic disturbance in brain tissue The Addisonian presents a pattern which is duplicated in our experience only in certain hypothyroid patients *This* is characterized by a) the presence of oscillations which are slower than the normal alpha rhythm, have a predilection for the frontal area of the cerebral *cortex, and are relatively refractory* to the usual effect of opening the eyes b) an unusual sensitivity of the electroencephalogram to voluntary hyperventilation, and c) a *reduction in incidence* of low-

Gaunt I don't know

van Dyke It would seem that the continued liberation of antidiuretic principle despite a fall in the osmotically active constituents of plasma implies a derangement of the neurohypophysial system

Gaunt It would seem to me that the point we mentioned would not explain all that occurs I don't know

van Dyke Certainly important information concerning the source of the antidiuretic substance of adrenalectomized animals could be obtained after removal of the adrenals from animals with diabetes insipidus

Gaunt We borrowed a few diabetes insipidus animals from Dr Pitts while he was in Syracuse to start a collaborative project on that Dr Birnie and Dr Capps did this work and what little data they obtained indicated that the serum of diabetes insipidus dogs contained less antidiuretic activity than normal

Shorr Would you refresh me Dr Gaunt about the specificity of the inactivation mechanism as far as general tissues in the body are concerned? Is it confined to the liver?

Gaunt No It is also present in whole blood and skeletal muscle for instance It may well be present in all tissues

Shorr In the kidney?

Gaunt Yes but the liver is most effective

Thorn We have carried on one experiment which might be of interest in relation to the possible site of action of the antagonism between cortisone and posterior pituitary antidiuretic activity In a patient with diabetes insipidus given pitressin cortisone failed to inhibit the antidiuretic activity of the latter Although obviously one should not attach significance to a single experiment it does suggest the fact that the most likely site of action of cortisone in inhibiting antidiuretic activity is at the hypothalamic or posterior pituitary level

Ralli We have an unusual case — a patient with diabetes insipidus due to a chromophobic tumor of the pituitary — who after eighteen months of observation developed adrenal cortical insufficiency and went into Addisonian crisis Prior to the development of the adrenal insufficiency we studied the effect of injecting pitressin in this patient on the excretion of antidiuretic substance A normal subject was

studied at the same time The antidiuretic assays were done by the method of Burn(39) in rats hydrated to 5 percent of their body weight by stomach tube One milliliter of the urine to be tested was injected intraperitoneally The rats in groups of four were placed in metal cages over a glass funnel and urine was collected directly into graduated cylinders The volume of urine excreted was noted every thirty minutes The results are shown in the two figures Figure 5 gives the results in the normal subject the clear circles show the effect of the subject's urine collected prior to the injection of pitressin and there was no antidiuretic effect The solid circles show the antidiuretic effect of the urine two hours after the injection of pitressin This was equivalent to the effect of 5 mU of pitressin (crosses) Concentrating the urine to one half of its original volume did not increase the antidiuretic effect (triangles) but did increase the chloruretic effect the figures for chloride excretion by the rats is given in the figure Figure 6 shows the results of injecting the urine from the patient with diabetes insipidus Three urine samples were assayed The clear circles are the assay of the urine sample voided at 7 o clock in the morning the patient

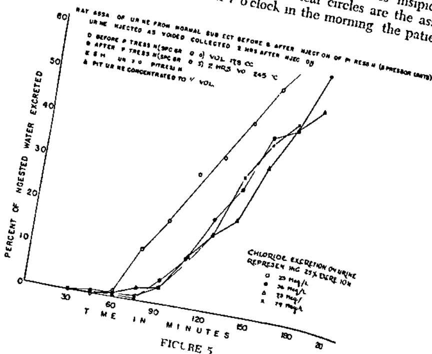


FIGURE 5

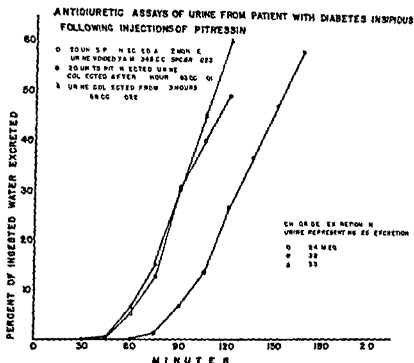


FIGURE 6

having taken 20 pressor units of pitressin at midnight The solid circles represent the assay of the urine voided 1 hour after the injection of a second dose of 20 pressor units of pitressin The triangles are the assay of the urine voided from the first to the third hour after this dose of pitressin It is interesting that the urine sample that remained in the bladder overnight had no antidiuretic effect nor did the sample voided in the second and third hours after another dose of pitressin The urine sample voided in the first hour after pitressin was injected had a marked antidiuretic effect One may infer that whatever pitressin is excreted in the urine is excreted principally within the first hour after the injection and that if the urine remains in the bladder the antidiuretic principle excreted is destroyed This patient with diabetes insipidus has now also adrenal cortical insufficiency and has been in Addisonian crisis three times The serum sodium has been at very low levels and he has required very large doses of whole adrenal cortical extract and salt to return him to sodium balance He is now controlled on hypoadrenal extract DCA salt about 16 gm daily and pitressin

every other night He always excretes water — even during periods of crisis

Shannon I am a little surprised to see a group of people interested in renal physiology sit around a table as they have this morning without protesting results presented which represent overall effects involving the animal as a whole particularly when the experimental preparations are difficult if not impossible to control and when the animal utilized in some experimental preparations has not been studied in order to determine the extent to which mechanisms utilized in the formation of urine are under specific nervous and/or humoral control All of us who have worked in the field of renal physiology appreciate that one can get the same overall net effect by a variety of combinations of mechanisms Consequently it is disturbing to me to see net effects utilized in an attempt to interpret the underlying mechanisms involved in the net effect without measuring specific variables Such experiments appear to me to have the nature of pilot experiments which can be used in the design of a more definitive type experimental approach

Thorn Dr Shannon's criticism is a good one On the other hand one must remember the very abnormal situations which surround an attempt to set up definitive studies in which only one variable is accepted We all realize that the overall retention of 50 mEq of sodium per day in a careful balance study may represent a very significant change in the individual's response to a hormone preparation although the sensitivity of the clearance technique is not great enough to detect this change

Shannon It might be helpful in order to clarify my point of view to describe very briefly two sets of experiments performed upon the dog In each case there was a dramatic increase in the renal excretion of water and in each the urine was characterized by sodium concentrations which were low as compared to plasma

The first observations were obtained in a study of a limited number of dogs with diabetes insipidus as the result of the more or less complete ablation of posterior pituitary tissue These animals weighing from 7 to 12 kilos were characterized by urine flows in the order of 3 to 4 liters per day with free access to water glomerular filtration rates and renal plasma flows were in low normal ranges When dilute saline (0 5 percent NaCl) was the only fluid available for drinking a sequence of events occurred wherein there occurred a dramatic increase in urine flow to a range of 12 to 18

liters per day Sodium concentration of the urine in the latter condition was essentially that of the ingested fluid(40,41)

An examination of the experimental data collected in these experiments suggests that following the beginning ingestion of the saline there were progressive increases in the amount of sodium localized in the body, progressive increases in the volume of extracellular fluid, accompanied by a progressive expansion of plasma volume Presumably as a result of the latter there were increases in glomerular filtration rate of roughly 100 percent as compared to the control levels The physiological defect which permitted this sequence of events was possibly due in part to an enhanced ability on the part of the renal tubules to reabsorb sodium and certainly to a diminished ability to reabsorb water against a concentration gradient

The second set of observations were those reported by Ragan Ferrebee Phyfe Atchley, and Loeb(42) wherein a syndrome of polydipsia and polyuria was produced by the administration of DCA and the polyuria enhanced by the further addition of sodium chloride in the diet

The detailed data presented in relation to the latter studies give little indication of any marked retention of sodium and a concurrent expansion of extracellular fluid and plasma volume, although it is possible that these things did occur to a minor degree In any case, the initial polyuria in the diabetes insipidus animal was due to a specific absence of posterior pituitary hormones, and the enhancement of the polyuria was due to the administration of sodium and the physiological consequences of the primary deficiency Polyuria in the latter series of observations was presumably due to the specific administration of DCA and its effect in enhancing sodium retention the latter having more dramatic effects when the sodium intake was high than when the sodium intake was low

Qualitatively then one must conclude that the same net effect is obtainable from the administration of sodium in the diabetes insipidus animal, on the one hand, and the normal animal receiving DCA, on the other There is no necessary relationship between the two experimental preparations other than the limited number of mechanisms available to the kidney with which to accomplish a physiological task Consequently, obtaining the same net effect under two different circumstances can by no means be taken as an indication that the same primary controlling factors are involved in producing a given experimental effect

There is little information available on the rat which will permit the definition of how this species accomplishes overall salt and water adjustment in terms of the discrete renal mechanisms which are involved We do have a fair amount of information about the human The human has a rather rigid rate of glomerular filtration When isotonic salt solution is administered there is little change in glomerular filtration rate and little immediate change in the amount of sodium excreted It would appear that the human organism does not have the capacity to increase salt and water excretion acutely as a result of a simple expansion of extracellular fluid It could be that this results from an inability on the part of the human to expand glomerular filtration rate

Janeway I think some experiments that Dr Gamble and Dr Wallace(43) did in children during the last two or three years showed that it takes about four days to reach equilibrium as you described for sodium salts On the fourth day they begin to excrete the excess sodium

Shannon We have comparable studies in the young adult

Dock The dog will come into equilibrium very quickly

Shannon Yes quite quickly

Thorn Why do you state that in the intact animal one can disregard the possibility of reciprocal changes in posterior pituitary secretion in response to salt loading?

Shannon I do not mean to say that the two preparations increase urine flow by two completely different mechanisms Rather as the result of two distinct physiological variables the same net renal effect is obtained

Large doses of DCA to a dog do not consistently produce a diabetes insipidus like state Nonetheless one must suppose that there is an interplay of various hormones as the result of DCA administration The diabetes insipidus only appears consistently when one increases the salt intake above normal One could argue

tonicity of the fluid reaching the gland From another point of view it would be unlikely to suppose that the simple absence of posterior pituitary as in the diabetes insipidus animal would produce a marked increase in salt retaining hormones under conditions of salt

loading since the physiological defect in diabetes insipidus animals is apparent with very moderate salt loads

Dock The salt load is always given with a lot of water

Shannon The experiments I have described were all with hypotonic saline The animal comes quickly into equilibrium in such a situation If the normal dog is loaded with isotonic saline and not too rapidly he also will come into equilibrium rather rapidly whereas the normal human does not come into equilibrium in any measurable period of time during an acute experiment

Gaunt There is no question about the limitations of the rat for detailed considerations of pure renal physiology I tried to make it clear in my introductory remarks that we at least had not attempted any detailed analyses of renal function in these various endocrine imbalances I think it could be done if that were one s primary interest For other aspects of the problem the rat can supply useful information with great ease and economy

Bott I should like to know how much water balance varies from animal to animal and from species to species I received the impression that adrenalectomized rats reabsorb more water from the tubules than do normal ones Is this due to a difference in the state of hydration? The blood volume is low in the adrenalectomized rat isn t it *Dr Gaunt*?

Gaunt Yes If one follows daily water intake in the adrenalectomized animal during the time when the animal is relatively healthy there is a higher than normal urine volume In some cases a negative water balance Adrenalectomized animals can excrete water in higher than normal amounts What they cannot do is to increase their urine volume in response to a water load anything like normal animals They will get rid of it in twenty four hours not in two

As to the state of dehydration that exists in the animal as a whole Dr Darrow has done work on that(44) I believe he found that there was no difference from normal in the total body water of adrenalectomized rats calculated on a fat free basis Such tissues as skin and muscle show a higher than normal water content There is extracellular dehydration and intracellular hydration

Thorn There is one important point which Dr Shannon has brought up in relation to the great load of sodium chloride which the dog can tolerate It might be that the large intake of dietary potassium ingested by dogs and their large potassium reserves

might be important factors in enhancing this apparent species difference

Pitts I think there is real species difference here at least in a quantitative sense Ladd and Raisz(45) demonstrated most convincingly that the normal dog can tolerate a phenomenal amount of sodium chloride in the diet without appreciable gain in body weight As salt load increases, glomerular filtration increases and the animal attains salt balance by excreting a fair proportion of the extra filtered load The situation in man is at least quantitatively different We studied some ten convalescent patients loaded with 27 gm of sodium chloride intravenously each day for three days The salt was administered as 3 liters of isotonic saline Before during and after this loading procedure we studied filtration rate and renal blood flow These patients came in to equilibrium with the elevated salt intake after three or four days with a weight gain of 5 to 18 lbs These patients did not show any uniform and certainly no impressive increase in filtration rate or renal blood flow I would infer that the greater capacity of the dog to handle sodium loads with minimal weight gain is in some way related to the capacity of this animal to increase the filtered sodium load

Burnett Yet in some humans the filtration rate is raised by acute loading

Pitts We gave five patients 1500 ml of 0 9 percent saline in thirty minutes at a rate of 50 ml per minute Clearances were measured before during and after this acute loading procedure Not infrequently did we find a fall in filtration rate during the infusion no rise afterwards yet a fairly significant increase in sodium excretion

Burnett Hypertonic?

Pitts Isotonic

Shannon We have done much the same experiment only giving 3 liters in the course of a comparable period of time That is about twice the rate of Dr Pitts experiments Towards the end of saline administration one observes a slight disturbance in renal plasma flow and glomerular filtration rate but this is transient Shortly thereafter these functions have returned to the control level

Burnett Administration of hypertonic saline solution has inconstantly caused increases in glomerular filtration rates in our hands yet Dr Leiter and Dr McCance have been able to increase filtration rate with hypertonic saline solutions(46 47)

Pitts I have taken hypertonic sodium bicarbonate intravenously and my filtration was raised roughly 20 percent There is I believe a considerable variability in man in the extent that filtration rate is raised by the infusion of isotonic and hypertonic solutions A moderate increase in sodium excretion results whether or not an increase in filtration rate occurs It is true that excretion is greater if filtration rate increases

Shannon The dog also has the capacity to retain both water and salt beyond that of a human He has a much more labile system Some of the observations on children by Farr seem to indicate certain cases of nephrosis are characterized by a much greater lability of the glomerular apparatus than there is commonly found in the normal adult To my knowledge a comparable study of the normal child has not been made

Burnett I don t know of any work on the normal child

Janeway Dr Metcoff(48) has contrasted salt loading in acute experiments in nephrotic children and normal children The loading has been incident to the measurement of renal clearances In the two groups there are big differences in how the sodium load is handled in the nephrotics a great deal of it is diverted extra renally the blood sodium rises and there is an actual increase in potassium excretion in the normal child most of the sodium load is excreted without a rise in blood sodium Dr Gladys Fashena* has shown similar responses to large doses of sodium chloride in nephrotics

Burnett We have found it true that administration of hypertonic sodium bicarbonate results in a rather striking excretion of potassium in the adult patient with nephrosis(49)

Lauson The lability of the nephrotic s clearances with changes in protein intake is particularly marked in the super normals those whose clearances are relatively super normal are more apt to have marked depressions if put on very low protein intake Sometimes this is not the case we had one careful experiment in a five year old child in which changing his protein intake from 50 to less than 20 gm of protein per day had no effect at all on around the clock creatinine clearances† Furthermore I think it would not be

* Personal communication

† This experiment and those referred to below were carried out at the Hospital of the Rockefeller Institute for Medical Research in the Department of Dr D D Van Slyke by Drs F P Chinard H A Eder R L Greif A Hiller G C Cotzias and H D Lauson

difficult to demonstrate this lability in some patients whose filtration rate on an ordinary diet is subnormal The human being certainly can change his glomerular filtration rate or urea clearance at least by means of drastic changes in protein intake even in the adult One experiment that I have in mind is that of Longley in Cleveland(50) who by greatly restricting protein intake reduced his urea clearances I believe by 30 percent or more It is necessary to reduce intake drastically to get much of a decrease On very high protein intake the urea clearance did not increase very much I would like to return to Dr Pitts experiments on man in which he failed to find a uniform increase in glomerular filtration rate Do you mean Dr Pitts that after equilibration occurred following the infusion the glomerular filtration rate did not increase even by a little or were the experiments confined to measurements made each day *during* the infusion of the material?

Pitts There were two different types of experiments In the first type filtration rate and blood flow were measured prior to and after three days of a hydrating procedure consisting of the intravenous administration of 3 liters a day of isotonic saline Subsequently the individual was dehydrated by the daily administration of 2 ml of mercuhydrin for three days and the measurements were again made In the second type of experiment filtration rate and blood flow were measured before during and after the administration of 1500 ml of saline at a rate of 30 ml per minute Both times

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would conclude that there is a quantitative species difference in the way the dog and man react to hydration and dehydration

Lawson I remember the experiment of Stewart and Rourke(51) which is graphed in Gamble's book on extracellular fluid I think it would be desirable to repeat that same experiment They had a patient presumably normal who had undergone minor surgery in whom four or five liters of isotonic saline a day produced a seven liter gain in extracellular fluid and eventual equilibrium of output to daily intake of salt I have always felt Dr Shannon that until proved otherwise this observation was at least qualitatively similar to your dog experiment(40) except that unfortunately the filtration rate was not measured by Stewart and Rourke I wonder if you would expect filtration rate to increase somewhat after saline retention had become really substantial as in their patient?

Shannon Our experiments simply indicate that the normal human in a chronic balance experiment can be expected to handle different salt loads without a measurable difference in glomerular filtration rate as conventionally measured

Pitts What prompted us to do our experiments was Ladd and Raisz (45) implication that conclusions reached in studies of electrolyte excretion in the dog could not be applied to man At the outset I disagreed with this view After finishing our studies I have changed my mind somewhat The relatively slight changes in filtration rate and blood flow in man in comparison with the large changes in the dog are such that they shake my faith in the safety of transferring too completely from dog to man concepts of the relative importance of one mechanism versus another

Fremont Smith Is there any evidence that urine volume is ever directly and primarily related to changes in glomerular filtration? It seems to me from all of the evidence I have seen whatever the glomerular filtration is doing that the change in volume is primarily related to reabsorption phenomenon

Shannon It is six of one and half a dozen of the other You have two processes going on all the time Filtration presents a volume of fluid to the tubular system which then reabsorbs the vast majority of solute and solvent in normal circumstances It is only when there is a defect in the system that a relationship can be demonstrated between the two at normal salt and water intakes The diabetes insipidus animal of about 10 kilos will excrete in the order of 5 liters a day However this level of urinary excretion can be markedly reduced as the result of severe restriction on water and this is usually accompanied or made possible by a marked lowering of glomerular filtration rate

Fremont Smith But also even greater change in his tubular reabsorption?

Shannon No sir

Fremont Smith Am I wrong? There is no change in the tubular reabsorption?

Shannon As far as sodium is concerned it is almost complete in the diabetes insipidus animal in either state

Fremont Smith I meant of water

Shannon This is apparently dominated by presence or absence of the antidiuretic hormone in the normally hydrated animal

Burnett You think the cutback in your animals was due to a decrease in filtration rate?

Shannon In that particular experiment I would assume that this is most likely One must appreciate that it is very difficult to obtain an animal completely devoid of all tissue capable of elaborating the antidiuretic hormone

Lauson In those experiments would you be willing to say flatly that the increased percentage reabsorption of that diminished filtrate volume represented an intrinsic tubular adjustment possibly independent of hormones?

Shannon I would not say that I don't believe one can be certain in any experimental preparation that one has completely ablated the tissue from which the antidiuretic hormone can arise

Lauson I wonder if the converse experiment that you reported in the second paper on the control of water excretion(41) doesn't also indicate a dependence of urine flow on filtration rate In this experiment you had a diabetes insipidus dog on a constant infusion of 20 mU per hour of pituitrin, which was more than adequate to reduce urine flow to a minimum when the filtration rate was about 25 ml per minute Then when you gave a large dose of water by stomach tube actual diuresis occurred in spite of continuation of the infusion of excess pituitrin Is this diuresis not then attributable to the fact that the filtration rate simultaneously rose from 25 to around 36 to 38 ml a minute? Is this not a clearcut converse of the other experiment, in which simple dehydration in the diabetes insipidus dog led to increased percentage reabsorption of water by the tubules in association with a reduced filtration rate?

Shannon I suspect so, although not published in the series other experiments were performed on the dog with isotonic sodium chloride being administered to produce an expansion of 5 to 10 percent of extracellular body fluid The pattern of response of the diabetes insipidus dog receiving what one might consider normal amounts of antidiuretic hormone was essentially the same as that of the normal

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ANTIDIURETIC FACTORS

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ANTIDIURETIC FACTORS may be defined as substances which reduce diuresis by water Depending upon their action they may belong to one of three groups of substances First a factor may be identical with neurohypophysial antidiuretic hormone This is probably the factor which is found in the urine of mammals after dehydration or after osmotic stimulation of the cerebrum Secondly, a factor may cause the liberation of antidiuretic hormone as is the case with several drugs such as acetylcholine or yohimbine Thirdly, the antidiuretic effect of a factor may have no relationship to the neurohypophysial secretion Such a factor may reduce urine flow by reducing the rate of renal plasma flow or the glomerular filtration rate or both A substance in this category could conceivably have an extra renal action No antidiuretic factor resembling closely the neurohypophysial antidiuretic hormone in its action on the kidney but not originating in the neurohypophysis has been discovered It is natural that antidiuretic factors especially those found in body fluids or secretions are often thought to be identical with the neurohypophysial secretion Therefore in the discussion which follows I shall speak briefly of the physiology of the neurohypophysis The assay of an antidiuretic factor is of such great importance for its characterization that I shall deal with this topic in some detail Lastly I shall discuss various antidiuretic factors which have been reported

The experiments of Ranson and his colleagues placed great emphasis on the physiological unity of the neurohypophysis consisting of the paired hypothalamic nuclei (especially the supraoptic and paraventricular nuclei) the infundibulum or stalk and the infundibular process or neural lobe of the pituitary Today most investigators agree that the structures comprising this physiological unit must be intact for the normal secretion of antidiuretic hormone in response to a physiological demand The signal for secretion may be released by a rise in the osmotic pressure of plasma Nearly all the antidiuretic hormone of the neurohypophysis is stored in the infundibular process which in the adult man contains about

15,000 mU The size of this stored secretion in terms of its activity can be appreciated from the human experiments of Barclay and Cooke(1) who demonstrated antidiuretic effects from intravenous doses of 0 5 to 2 mU

A distinction must be made between the antidiuretic principle and the protein of the posterior pituitary which was isolated by van Dyke *et al* (2) This protein contained both the antidiuretic and the oxytocic principles and had a potency corresponding to 61 micrograms per unit of standard powder Under discussion today is the antidiuretic principle which possibly is a fragment of a large protein molecule The most potent extract reported contained a unit in about 2 micrograms (0 002 mg) Perhaps its true potency is as great as the pure or nearly pure oxytocic principle of Pierce and du Vigneaud(3) whose product was nearly twice as potent in terms of oxytocic assay The antidiuretic principle is dialyzable and ultrafiltrable It is readily adsorbed in blood and on numerous other substances such as artificial zeolites It resists digestion by pepsin but is readily inactivated by trypsin Heat as well as high or low hydrogen ion concentrations destroy it Apparently the -S S-group of cystine must be intact if its activity is to be retained This amino acid is an important constituent both of the antidiuretic principle and of the hypothetical parent protein

In lowering the rate of urine formation by purely renal mechanisms, antidiuretic hormone increases the renal reabsorption of water Most investigators have concluded that it has no effect on the glomerular filtration rate However Barclay and Cooke(1) in their recently described human experiments, observed that in some of the subjects the antidiuretic effect may be accompanied by reduction of the glomerular filtration rate, in fact in five of thirteen subjects, there was a reduction of both the glomerular filtration rate and the rate of renal plasma flow Barclay and Cooke used small doses totaling 0 5 to 2 mU administered as 2 to 80 microunits per minute during or after such doses they observed antidiuresis in each of thirteen subjects It is not surprising that both glomerular filtration rate and the rate of renal plasma flow have been reduced in association with antidiuresis after the enormous doses which have been used in either animals or man As recently as 1947 Little and his colleagues(4) injected 10 units of pitressin intramuscularly into the human subjects under study So far as the normal rate of secretion is concerned there is good

agreement at least in the dog that the rate is about 1 to 5 mU per hour per dog(5 6)

Antidiuretic hormone is an example of a stored internal secretion which is liberated according to the body s needs and in this respect resembles the stored secretion of the thyroid in contrast with adrenal cortical secretion which is rapidly but temporarily elaborated according to the demands of stress The control of the liberation of antidiuretic hormone is principally effected by the osmoreceptors associated with the neurohypophysis The connections with other parts of the central nervous system must be numerous although it is not possible to give any accurate picture of the relationships In what form the hormone is liberated is unknown It can be detected in the internal jugular blood after osmotic stimulation An unknown fraction is excreted in the urine Presumably in the interval between liberation into the blood stream and excretion in the urine a considerable part of the hormone is inactivated by reducing substances and enzymes such as proteinases and polypeptidases After enormous intravenous doses 10 to 25 percent of the dose may be excreted in the urine The endogenously secreted hormone which appears in the urine under goes sedimentation in the ultracentrifuge like a large particle This urinary hormone easily undergoes fragmentation with liberation of the nonsedimentable antidiuretic principle(7)

Much effort has been expended in attempts to demonstrate antidiuretic activity in various body fluids The hormone is not present in cerebrospinal fluid despite early suggestions to the contrary The hormone can be detected in the blood of the dog after osmotic stimulation and in the serum of rats especially after adrenalectomy Owing to its relatively simple composition the urine is perhaps the best fluid in which to demonstrate the hormone unequivocally In man the maximum concentration appears to be 1 to 2 mU per milliliter In other mammals with maximum osmotic stimulation the greatest concentration is about 6 mU per milliliter (dog and rat) In a desert rodent the kangaroo rat (*Dipodomys merriami*) nearly ten times as high a concentration has been found(8)

Any study of antidiuretic factors in body fluids has to take into account the great difficulties of assay Without accurate and quantitatively acceptable assays no conclusion can be reached concerning the alleged presence of true antidiuretic hormone For example chloruresis is sometimes accepted as a necessary feature of the action of the hormone and may be the basis for denying its

neurohypophysial origin Yet a careful survey of the literature does not support this conclusion and it appears certain that depending upon conditions, an increased rate of excretion of chloride or of cations such as sodium or potassium does not necessarily occur after the administration of the principle

Anslow and his colleagues(9) concluded that an effect on electrolyte excretion depends upon the water load Their experiments suggest that without suppression of the endogenously secreted hormone an effect will not be observed Although Shannon(5) believed that the principles administered as pituitrin" inhibited the tubular reabsorption of chloride, he never observed this effect in one of the four dogs with diabetes insipidus which were used for his experiments Other experiments have been performed by Sartorius and Roberts(10) in the dog and they concluded that whether or not hydration was present the intravenous injection of the antidiuretic principle increased the rate of excretion of sodium and that this effect was antagonized by desoxycorticosterone acetate Human experiments have been reported by Hart and Verney(11), McQuarrie and co workers(12), White and Findley(13), Barclay and Cooke(1) and others From these observations it may be concluded that in man the antidiuretic principle has no consistent effect on the rate of excretion of chloride

Some workers have attributed a chloruretic effect to the oxytocic principle (Fraser(14), Kuschinsky and Bundschuh(15), Dicker and Heller(16), Heller and Stephenson(17) and Schaumann(18, 19) Some of the later work led to the conclusion that the primary effect is on cations and that chloride was the accompanying anion It appears valid to conclude that many factors modify electrolyte excretion whether antidiuretic principle or oxytocic principle or both be administered Among these are the electrolyte metabolism the dietary electrolyte and possibly the rate of secretion of adrenal cortical hormone Chloruresis and an increased excretion of sodium or potassium or both are not necessarily characteristic of the action of the antidiuretic principle

The principal factors in the bioassay of antidiuretic hormone are specificity, sensitivity and quantitative precision Pertinent examples are summarized in Table I The hydrated dog receiving a body fluid for assay is considered to be particularly appropriate, especially the dog with diabetes insipidus Particularly to be avoided is the comparison of an intravenous assay in one species with subcutaneous or intraperitoneal assay in another In all instances

material under examination should be assayed by the same method, thus avoiding the error into which Heller(20) fell in believing that he had demonstrated separate antidiuretic and pressor hormones Fraser(21) was able to point out that when all of Heller's extracts were assayed intravenously they did not differ in their antidiuretic and pressor effects The rat receiving an extract subcutaneously may absorb hormone slowly owing to contaminating substances so that the apparent titer of potency may be deceptively high Noble Plunkett and Taylor(22) stated that intraperitoneal injection into rats may provoke an antidiuretic effect from toxic materials

TABLE I

METHODS OF ASSAYING ANTIDIURETIC PRINCIPLE

Animal	Route	Specificity	Sensitivity*	Quantitative precis on
Rat	Subcut	Imperfect	1 mU /100 gm	Poor with extracts
"	Intraper	"	2 mU /100 gm	Better than Subcut
'	Intraven	'	0 02 mU /rat	Good
Rabbit	Intraven	Poor?	0 5 mU /rabbit	Fair?
Dog	Intraven	Imperfect	0 25 mU /dog	Fair
" (diabetes insipidus)	Intraven	Best	0 25 mU /dog	Fair

*Optimum doses for quantitative estimates are higher

The following considerations suggest that intravenous *assay in dogs heavily hydrated is especially valuable* a) *the assay animal* is so sensitive that no chemical manipulation of the body fluid is necessary before the intravenous injection, b) if there is a gross renal effect, such as an interference with glomerular filtration rate this can be readily detected by creatinine clearances or, less exactly, by determining the rate of excretion of endogenous creatinine, c) inactivation of the hypothetical hormone can easily be under taken *with a reducing agent such as sodium thioglycolate Usually* there is a lag of a few minutes in the antidiuretic effect of true hormone If sympathetic vasoconstrictors are stimulated there is *a rapid supression of* the flow of urine Precautions also have to be taken to avoid an unusual release of neurohumoral *transmitting* agents affecting neurohypophysial secretion For example O'Connor and Verney(23) found that epinephrine can *interfere* with the release of antidiuretic hormone Pickford's numerous *experi ments*(24) support her contention that acetylcholine acts on the

neurons of the supraoptic nuclei thus provoking a liberation of antidiuretic hormone

The possible types of antidiuretic factors were discussed in the first paragraph of this communication and will again be mentioned briefly here The specific factor is derived from the neurohypophysis and acts directly on the kidney usually with little interference with glomerular filtration rate or the rate of plasma flow Some substances are thought to stimulate the neurohypophysis so that the hormone is liberated In this group belong acetylcholine and the derivatives of phenylcinchoninic acid studied by Marshall and his colleagues(25 26) * No antidiuretic substance physiologically resembling the true hormone but not derived from the neuro hypophysis has been discovered Lastly a toxic effect of an unknown substance may provoke an apparent antidiuresis owing to inter ference with the circulation of the kidney either locally or by general cardiovascular effects

In Table II are presented the drugs which have been discovered to have an antidiuretic effect Their mechanism of action is usually to produce a liberation of the neurohypophysial hormone

When antidiuretic hormone has been thought to have been identified in the urine various alternative interpretations are pos sible Usually one of the following hypotheses has been adopted on the assumption that true antidiuretic hormone has been detected a) the rate of liberation of the hormone is increased b) the rate of destruction of the hormone has been reduced as in hepatic disease c) there is a hormonal imbalance so that the rate of liberation is altered as when there is a deficiency of adrenal cortical secretion(38) d) true antidiuretic hormone is not present but its identification is an assay artifact and e) an abnormality of the kidney permits exceptional amounts of the hormone to appear in the urine It is not possible to speculate on the rate of secretion of true antidiuretic hormone from the amounts detected in the urine All the observations have been made with great quanti ties (e g 2 000 to 5 000 mU injected intravenously into man by Taylor and Noble(39)) after such enormous doses in both men and animals 5 to 23 percent of the injected dose have been recovered When injections are made subcutaneously or intraperi toneally interference with absorption owing to a contaminating substance may yield an artificially high titer Hence intravenous

* Also by personal communication

TABLE II
DRUGS AS ANTIDIURETIC AGENTS

Drug	Antidiuretic effect in diabetes insipidus	Investigators
Acetylcholine	No	Pickford(24), others
Yohimbine	"	Zunz(27), Fugo(28)
Morphine*	?	de Bodo(31)
Phenobarbital	No	de Bodo(32)
Nicotine†	"	Burn *et al* (34)
Dimercaprol (BAL)	'	Earle and Berliner(35)
3 hydroxy 2 phenyl cinchoninic acid and related compounds	Probably No	Marshall and Blanchard(25)
Choline	No expmts reported	Molitor and Pick(36)
Atropine	" " "	Cow(37), others

*Also reported to reduce rates of GF and RPF (Handley and Keller(29)), (Brown, Hodges and Bradbury(30))
†Antidiuretic effect may be absent in nonsmokers (Eggleton(33))

assays in the dog as used by Hare and his co workers and by Ames Moore and van Dyke appear to yield the most reliable information

The first unequivocal demonstration of antidiuretic hormone in urine was made by Gilman and Goodman in 1937(40) Other experiments employing the rat have been performed by Robinson and Farr(41) as well as by a number of later investigators Usually the urine was prepared for injection by concentrating it and then dialyzing the concentrate To circumvent this procedure by which the final extract may contain toxic material attempts have been made to adsorb the hormone and later to elute it The first of these methods was that of Noble Rinderknecht and Williams(42) who used zinc ferrocyanide as the adsorbent eluted the hormone with ammonia ethanol and then fractionated the eluted material with ethanol Two examples of the latest methods in which urine has been the starting material are those of Stueck Leslie and Ralli(43) who adsorbed the hormone from concentrated urine on permutit and accomplished elution by 5 percent sodium chloride in 1 molar acetic acid In the same year Grollman and Woods(44) described adsorption on charcoal with elution by glacial acetic acid and precipitation of the active material by a mixture of equal parts of absolute ethanol and petroleum ether

An increase in antidiuretic factor usually detected in urine and usually considered of neurohypophysial origin has been described for a number of clinical conditions The report of Robinson and Farr(41) correlated a high urinary antidiuretic titer with clinical edema in the premenstruum nephrosis and Cushing s disease Toxemias of pregnancy have been thought to be accompanied by an increased secretion of antidiuretic hormone(45 46 47 48) Stander and his colleagues questioned the neurohypophysial origin of the urinary principle Ham and Landis believed that it was derived from the placenta

According to Ellis and Grollman(49) antidiuretic principle is present in the urine of a large proportion of human subjects with hypertension (eleven of fifteen) but cannot be detected in the urine of normal persons (none of nine) Also these workers believed that hypertensive dogs excreted two to four times as much antidiuretic principle as normal dogs Experimental procedures in normal subjects may lead to an increased rate of secretion of antidiuretic principle Among these are dehydration electroconvulsant therapy and fainting The titers described by

Taylor and Noble(39) are unusually high and it seems certain that the authors meant to express their results in microunits rather than in milliunits

Especially favored for study is hepatic disease whether in experimental animals as in rats on a high fat diet or in man with severe hepatic disease such as cirrhosis with ascites In most instances the rat has been used for assay and there are unexplained inconsistencies in the reports which tend to nullify the conclusions (compare the results of Leslie and Ralli(50) with those of Hall Frame and Drill(51)) Human liver disease may be associated with a low rate of urinary excretion of water and with a considerable diuresis as ascites recedes An attractive hypothesis has been that of Ralli and her co workers in 1945(52) and later who postulated a decreased destruction of antidiuretic hormone in such subjects particularly in association with ascites This presumably in part would explain water retention Quantitatively there is a wide difference between their results and those of van Dyke Ames and Plough(53) who found by assays in dogs a slight but real increase in the titer of antidiuretic principle in the urine of persons with cirrhosis and ascites as compared with cirrhotic patients without ascites They never were able to detect the high titers reported by Ralli and her co workers who performed their assay in rats

Sims(54) also believed that infectious hepatitis could lead to a diminished destruction of antidiuretic hormone as revealed by increased urinary titers Sims likewise used rats for his assays

An antidiuretic effect due to the hormone relaxin has been described by Zarrow(55) He employed extracts of sow ovaries or pregnant rabbit blood and reported that the volume of urine excreted by rabbits is diminished after the injection of such extracts None of his observations justify his conclusion that such extracts induce water retention since he offers no record of the water intake of his animals The whole effect could be a toxic reduction in food and water consumption

Baez Mazur and Shorr(56) attributed the oliguria of cirrhosis infectious hepatitis nephrosis and congestive heart failure to the indirect effect of a liberation of their vasodepressor material (VDM) They considered VDM to be identical with ferritin or apoferritin and believed that either of these proteins acting on the neurohypophysis causes an increased liberation of antidiuretic hormone

SUMMARY

The possible origin and nature of antidiuretic factors are discussed with special consideration of the neurohypophysial antidiuretic hormone The difficulties of assay and of characterization of an antidiuretic substance are described Examples of antidiuretic factors such as drugs or constituents of urine are discussed and an evaluation of their significance is attempted

DISCUSSION

Lauson I think it might be of interest to mention some experiments that were done on me some years ago which were a direct imitation of Shannon's experiments(57) in which we infused pitressin at a constant rate while I was maximally hydrated The results indicated that my kidney sensitivity to pitressin was of the same order of magnitude that he had observed in the dog The creatinine U/P ratios achieved at the end of two hour infusions in me and after forty five minutes in Shannon's Dog D were approximately the same for the same rates of infusion when the latter were expressed as milliunits of pitressin per hour per kilo gram of body weight I might also point out that the lowest effective dose was as little as 7 5 mU per hour This rate reduced the urine flow to something over 1 ml a minute, not a maximal effect but quite substantial Therefore the extraordinary renal sensitivity to these extracts applies to man as well as to the dog

White In a series of perhaps a dozen normal human subjects the results were about the same In our experience 0 25 to 0 5 mU will give a good but not maximal antidiuretic response in any diabetic insipidus dog that we have used. The normal human subjects have shown about the same sensitivity on the basis of body weight, it takes about 1 to 3 mU per average size man to give about the same percentage drop in urine flow when he has been hydrated, putting out 5 to 6 ml a minute

Lauson As a single injection?

White As a single intravenous injection Normal young subjects were compared with normal old subjects no difference in sensitivity was found So the dose of pitressin for unmistakable threshold response is about 0 25 to 0 50 mU per 15 or 20 kilos of either dog or man Perhaps a third of the time you may get a barely perceptible

response with 0 1 mU in the dog with diabetes insipidus So I think there is enough evidence to indicate that the sensitivities of the dog and the man are about the same

Dock Your normal subjects here were all well hydrated?

van Dyke They were adequately hydrated, the specimens of urine were obtained in the morning before food or water

Dock They were not restricted the previous day?

van Dyke No

Dock In water restriction of man how high does the urinary concentration of ADH go?

van Dyke We have very little data ourselves The highest we have ever observed is about 2 mU equivalent of pitressin per milli liter of urine

Dock As high as any cirrhotics?

van Dyke I don't know how high cirrhotics go

Ralli We have assayed the urine of normal human subjects who have been dehydrated for forty eight hours and have obtained a very high antidiuretic titer

If I may, I should like to discuss some of the points mentioned by Dr van Dyke, but first I would like to explain how I happened into this field As you know it had been thought for many years that the determining factor in the control of ascites in patients with cirrhosis of the liver was the level of serum albumin My associates and I had been studying and treating a group of patients with cirrhosis and ascites and had determined the serum constituents including serum albumin at regular intervals of two to four week for periods of four to twelve months Interestingly enough we found that the serum albumin level *did not* increase before the ascites was controlled but rather that following diuresis and the associated decrease in ascites, the level of albumin in the serum rose gradually(58) In addition we observed as had been reported many times in the literature that the urine output in patients with liver disease was greatly reduced and that the twenty four hour output was often as low as 350 to 500 ml This suggested to us that the kidney might play an important role in the production of the antidiuretic state encountered in patients with cirrhosis and that this state of antidiuresis was a factor in the production of ascites The report by Robinson and Farr(41) on the presence of an anti-

diuretic substance in the urine of patients with edema stimulated us to do antidiuretic assays on the urines of patients with cirrhosis of the liver We found significant amounts of an antidiuretic substance in the urine of these patients, particularly when ascites was present(52) All assays were done by the method of Burn* in rats hydrated to 5 percent of their body weight by gavage We have continued to use this method since 1944 and we believe it to be a reliable method of assay as judged by control assays on known amounts of pitressin In continuing these studies we have attempted to determine the nature of the antidiuretic substance present in urine and in the course of this work have done innumerable assays of both urine and pitressin treated in a variety of ways An interesting species difference was observed in simultaneous assays done with pitressin in hydrated dogs as compared to rats Water diuresis was established in the dog by administering 75 to 150 ml of water per kilogram of body weight The material to be tested was given intravenously through an indwelling needle and injections of

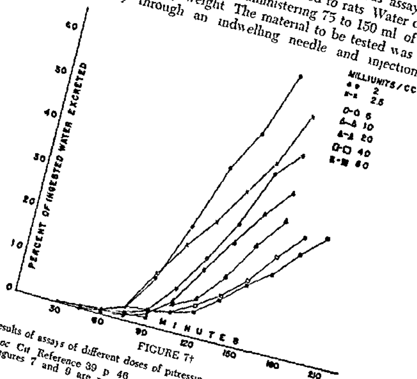


FIGURE 7†

Results of assays of different doses of pitressin in hydrated rats

*Loc Cit Reference 39 p 46

†Figures 7 and 9 are reprinted from Am J Physiol 163, No 1 141 147

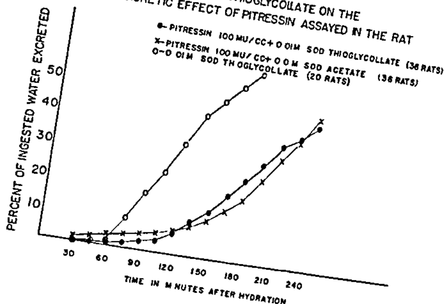


FIGURE 8

freshly diluted commercial pitressin* were given during the assa
experiments in order to quantitate the antidiuretic potency of the
treated pitressin We assayed commercial pitressin* as such in
varying dilutions dialyzed pitressin and pitressin treated with
sodium thioglycollate according to the method reported by Dr
van Dyke and his associates(7) The results are illustrated in
Figures 7 8 and 9 Figure 7 shows the antidiuretic response in
hydrated rats to doses of pitressin varying from 2 to 60 mU and
also shows the consistency of these assays in rats Figure 8 shows
the effect of treating pitressin with sodium thioglycollate and it is
quite clear that the treatment with thioglycollate did not inactivate
the antidiuretic effect of the pitressin in rats Figure 9 shows assays
of thioglycollate treated pitressin in the dog ar ' ' ~ the anti
diuretic activity was completely abolished
the result reported by Dr van Dyke It ical to
and the dog react to thioglycoll the rat
This and other exper sted to us itressin
activity of commerci n be fr liuretic
one substance one of when th
not active in the dog nts are rat l
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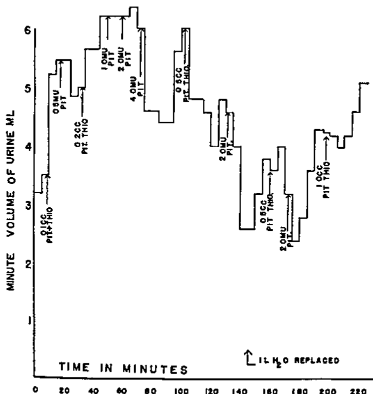


FIGURE 9

Antidiuretic effect of pitressin treated with 0.01 M sodium thioglycollate and pitressin treated with 0.01 M sodium acetate injected i.v. into a female dog during water diuresis

detail in a recent paper(58) As far as the antidiuretic substance in the urine of patients with liver disease is concerned, we have found the dog much less sensitive than the rat

Lauson What did the thioglycollate alone do in the rat?

Ralli Thioglycollate alone has no appreciable antidiuretic effect when injected intraperitoneally in the rat Whenever hydrated rats are given any solution intraperitoneally the rate of urine excretion is slightly less than if they were hydrated and no injection at all were given

van Dyke If you performed assays intravenously in the rat what would you expect?

ties along these lines Certainly if the dog gets large amounts of antidiuretic hormones he shows no such effect He merely decreases his water intake and gets along very well indeed, and I imagine most of the patients would have the intelligence of the dog insofar as regulating their water intake is concerned Surely they would not have much difficulty in getting rid of their electrolytes

Binkley What is the evidence that there is an enzyme in the liver that destroys the antidiuretic hormone?

Ralli I think Dr Gaunt could also discuss that question There were some experiments by Heller(59) in which pitressin was incubated with liver slices and they reported that the antidiuretic effect of the pitressin was destroyed I do not know if this has been confirmed There is no absolute evidence that the liver of the patient with cirrhosis is incapable of inactivating pitressin We have suggested this as a possibility but it remains to be proved

Gaunt I do not have the evidence at hand These were Dr Birnie's(60) experiments with which I had nothing to do He studied the effects of pH, heat, heavy metals, etc on the hepatic material and the evidence convinced him and others who reviewed it that he was dealing with an enzyme that inactivated pitressin I cannot elaborate the point except to say that in all of its chemical properties the material was identical to what has been called insulinase

Binkley The order of activity you gave for the concentration of enzyme is exactly the order of activity for concentration of free sulfhydryl in the liver suggesting that inactivation occurs as a result of combination with sulfhydryl groups rather than by enzymic activity

Ralli I would like to suggest another possible explanation for the impaired or disordered kidney function in the patient with severe liver damage Cirrhosis of the liver is a disease with widespread metabolic effects which involve the "nutritional" state of the tissue cells The kidney cells undoubtedly are also affected by this situation and the question arises as to whether the condition of the intracellular constituents of the kidney cells may not be affecting their physiological capacity to function normally

Bradley I would like to ask Dr Shannon if he believes antidiuretic hormone acts solely on water reabsorption

Shannon The facts are not available I believe that it is purely a matter of opinion and speculation First, a pure antidiuretic

hormone is not available Second the same preparation when used in four dogs may not produce identical results in all animals As Dr van Dyke has pointed out that is quite common in any one series of investigations If sodium excretion is plotted against dosage of antidiuretic hormone administered continuously in the range of 5 to 20 mU per hour there is no change in sodium excretion accompanying the change in water excretion At higher dosage some dogs progressively increase sodium excretion but on the basis of our evidence only when the hormone is administered above the physiological range

Ralli Is the relationship between hormone administration and sodium excretion roughly a logarithmic one?

Shannon The data would not permit that type of analysis

Dock Did the group in using the artificial kidney on patients with severe jaundice and anuria observe a marked rise in blood pressure and urine excretion after dialysis of the patient s blood?

Thorn Yes(62)

Dock This dialyzable shock factor can not be either pitressin or apoferritin neither of which is dialyzable?

Thorn No these molecules are too large to pass through our cellophane membrane

Dock Some substance of that sort may be in Dr Ralli s urine extracts or at any rate a substance not destroyed by reducing substances which inactivate pitressin It probably is quite different from the antidiuretic substances apoferritin and pitressin

Shorr At the risk of introducing another variable in the problem of diuresis I would like to present some of the results of our experiments on the antidiuretic effects of ferritin(56) Our early experiments were run with concentrates of the liver vasodepressor material VDM which though crude were pure enough to be injected intravenously The animals were given a continuous infusion of 5 percent dextrose until the urinary output became relatively constant At this point the addition of VDM to the infusion fluid caused a sharp curtailment of urine flow which persisted for some time after the infusion of dextrose alone was resumed

After the identification of VDM as ferritin or apoferritin these experiments were repeated using both oral and intravenous hydration Both ferritin and apoferritin were effective in amounts ranging from about 150 to 300 micrograms ferritin N per kilogram

less sensitive We have been able to utilize a combination of the immunochemical and mesoappendix methods to identify very small amounts of ferritin The reaction between antiferritin sera and such minute amounts of ferritin does not produce a visible precipitate but it does abolish the vasodepressor activity as measured by the mesoappendix test(63)

The liver possesses a very active mechanism for removing ferritin from the blood and inactivating it so that the amount of ferritin injected is much larger than the amount remaining in the blood stream to cause antidiuresis The discovery that ferritin exists in two forms gave us considerable understanding of this inactivation process The active form which produces a vasodepressor response in the rat mesoappendix test contains sulfhydryl groups When these groups are oxidized to the disulfide form the ferritin is inactivated In the normal liver ferritin is present largely if not entirely in the disulfide form The oxidation of sulfhydryl groups appears to take place readily in the normal liver This prompt and efficient inactivation results in a very low concentration of ferritin in the blood stream Rat bioassays of blood samples taken from a dog during an antidiuretic test revealed activities corresponding to approximately 0 001 to 0 002 microgram of ferritin N per milliliter In other words about 99 9 percent of the injected ferritin is immediately inactivated(64)

Janeway When you talk about active ferritin do you mean its vasodepressor activity?

Shorr Yes as measured by the rat mesoappendix test

Janeway It is vasodepressor activity?

Shorr Yes

Janeway The activity is present only when there are sulfhydryl groups and bears no relation to its ability to bind iron?

Shorr That is true but it is also interesting that treatment of disulfide ferritin with reduced glutathione or cysteine converts it to the sulfhydryl form and at the same time labilizes the ferritin iron converting it to the ferrous state(65)

Thorn Did you say the disulfide form has no antidiuretic effect?

Shorr We do not know We haven t enough data so far

The normal liver only releases reduced or active ferritin under anaerobic conditions In nutritional cirrhosis in rats the liver

metabolism is disturbed so that active ferritin is released under both aerobic and anaerobic conditions and as a result ferritin is constantly present in the blood stream It was therefore of interest to see whether or not the potential participation of ferritin in antidiuresis was supported by its presence in conditions associated with antidiuresis in man(66) We studied a group of eight cirrhotics with edema ascites and antidiuresis as measured by the Fremont Smith water test All had very considerable amounts of ferritin in the blood unopposed by the oppositely acting factor VEM Two other patients with cirrhosis but without edema and ascites and with normal water tests revealed only traces of ferritin The concentration of ferritin in the serum of patients with antidiuresis is of about the same order of magnitude as we found in the circulation of the dogs during antidiuretic experiments

In seven patients with the nephrotic syndrome ferritin was present not only in the plasma but also in the edema fluid Here too the oppositely acting VEM was not present We are very much indebted to Dr Lauson for permission to study one of his cases before during and after treatment with intravenous albumin There was a significant fall in ferritin activity in the blood during the albumin diuresis with a prompt return to the original high value on the re accumulation of edema following cessation of albumin therapy

Finally seven cases of congestive heart failure with edema and ascites were studied None of these cases had hypertension In all both plasma and edema fluid contained considerable amounts of ferritin without admixture with VEM

The difference between the results in these cases and in hypertensive patients is that in the latter both ferritin and VEM are present in the plasma in amounts which are approximately equivalent physiologically Rat tests of hypertensive plasmas are neutral or show a slight predominance of ferritin or VEM However treatment of the plasma to inactivate either ferritin or VEM always unmasks a considerable concentration of the oppositely acting factor Thus the possibility exists that these two factors with opposite actions on the blood vessels may also be oppositely acting as regards their effect on the posterior pituitary however our purification of VEM is not yet at a stage to permit us to test this hypothesis

We are still actively investigating the antidiuretic phenomena in the dog and are seeking more conclusive data to support our

hypothesis At this stage in our work it appears at least possible that ferritin may constitute another element participating in anti diuresis There are two mechanisms which could operate in these patients In congestive heart failure with edema hypoxia might lead to the continuous release of reduced ferritin from the liver Protein undernutrition which leads to the release of active ferritin in cirrhotic rats may be responsible for its occurrence in nephrosis and decompensated cirrhosis

Thorn You don t think that the response in a nephrotic is just an uncovering of ferritin by reduction in the VEM factor?

Shorr In nutritional cirrhosis in rats there is a renal defect as a result of which the kidney is not able to form VEM We are guessing that in nephrosis the same defect occurs

Thorn Our dialysis experiment would support your hypothesis In the patient in shock we apparently dialyze a factor which does allow the blood pressure to return to normal levels and to be maintained for four to six hours and then return toward a low level If the liver particularly has not improved in this time a second run of five or six hours of dialysis is done and the patient becomes responsive again to vasopressor stimuli The interesting thing along with Dr Shorr s concept is that the hypertensive does exactly the same thing When you dialyze a hypertensive patient s blood pressure goes ever so much higher than the patient with normal blood pressure suggesting that possibly a diffuse depressor substance is balanced at a high level and the fact is we don t know whether we are dialyzing off an activator of ferritin or an inactivator of renin system but the substance is diffusible and goes through a cellophane membrane

Shorr Ferritin is not diffusible

Thorn We have not been able to explain the diuresis We observed it in patients run without renal shutdown on the basis solely of improvement in blood pressure It has been pretty obvious from the start that something modified the diuresis or accelerated the diuresis in a patient who has been run on the kidney five or six hours if his kidney has capacity to respond

Dock The salt output goes up too?

Thorn Yes

Janeway Dr Shorr can you characterize VEM chemically?

Shorr I wish we could We are working on it actively now

Janeway It is not renin?

Shorr So far, it doesn t seem to be renin We don t think it will turn out to be hypertensin since it is not musculotropic

Lauson I would like to ask Dr Shorr if he has done any experiments in which homologous ferritin has been continuously administered to see if it is chronically antidiuretic

Shorr Homologous ferritins usually continue to be antidiuretic when repeatedly injected in dogs However out of a large series of dogs receiving homologous ferritins two no longer responded with antidiuresis We have no explanation at present for the failure in these two animals

White Is it your concept that the overproduction of posterior lobe hormone is causally related to any of these diseases?

Shorr If I am allowed to speculate at this stage in our knowledge of these factors I would postulate that in nephrosis cirrhosis and heart failure accompanied by edema the normal liver metabolism of ferritin is disturbed As a result of this defect active ferritin is released into the blood stream and accumulates in amounts which are of the same order of magnitude as those found in the circulation in dogs in the course of our antidiuretic experiments with injected ferritin From preliminary animal studies we infer that ferritin acts in some way upon the posterior pituitary causing release of antidiuretic factors from that gland It appears that the antidiuretic activity present in the urine in our animal experiments is not ferritin

White It might be worth while to mention here some observations bearing on the matter that was discussed earlier I know of two human subjects not suffering from diabetes insipidus who for a different reason were kept on large doses of pitressin or pituitrin one of them for over six months and one for about eighteen months with no retention of water or anything else Their intake was just cut down to balance their output and they got along perfectly well These were hypertensives the work was done to see whether they could be helped with more pitressin than they were making for themselves All that I am prepared to say – it was not my work – is that it did not hurt them at all

Shorr There is one other possible effect of the presence of ferritin in the blood stream It may bring about in the peripheral

vascular bed, a state of vasodepression with reduction of spontaneous vasomotion and relaxation of the precapillary sphincters When this occurs *the capillary bed becomes hyperemic with continuous* flow through many capillaries which ordinarily have only intermittent flow These changes would all favor hydrostatic rather than osmotic forces in the exchange of fluid between the capillaries and the tissues and might contribute to edema in that way Perhaps both effects on the pituitary and on the peripheral vascular bed are needed to produce edema

White That would seem to me more important than overproduction of posterior hormone

Lauson Would you say this circumstance would favor outward filtration rather than absorption?

Shorr We do not think of the capillary bed as consisting of inert endothelial tubes There are muscular units, the precapillary sphincters from which the true capillaries arise These are capable *of vasomotion by which the relative relations between osmotic* and hydrostatic forces may be altered according to need

Fremont Smith That means you have effective capillary filtration increase

Shorr Yes That would be in cases of edema

Fremont Smith As far as we know the effect of capillary pressure as measured has not been shown to be effective

White How often has it been satisfactorily measured?

Thorn Does the ferritin level of serum go down after ferritin extraction?

Shorr I cannot tell you

Janeway Do all capillaries behave the same in response to this material? *What about the capillaries in the kidney* itself?

Shorr We have made no direct observations on the capillary bed of the kidney but we would expect a variety of factors to operate Normally the renin hypertensin system might oppose the action of ferritin or VEM might have a local action However, under certain conditions the kidney cannot form VEM and conceivably the formation of renin may be similarly prevented In such instances the vasodepressor action of ferritin could predominate and we suspect this may have been the case in Dr Van Slyke's studies on

hemorrhagic shock He found a striking alteration in the filtration fraction which fell profoundly as irreversibility developed We have found hyporeactive shock to be associated with predominance of ferritin in the blood and with an inability of the kidney to form VEM The capacity of the kidney to form VEM is destroyed by prolonged hypoxia

I am wondering, Dr Gaunt, whether an observation we have made may have a bearing on your problem When we assayed the blood of a few adrenalectomized animals after withdrawal of DCA we found ferritin predominance If this is confirmed in a larger series it might be at least a contributing factor in the subsequent antidiuretic state

Thorn But DCA in man does not correct the excessive antidiuretic effect

Shorr That is right I had in mind the measurable antidiuretic factor in such animals

Gaunt DCA in doses which do not repair the deficient diuretic response to water will reduce the concentrations of serum antidiuretic substance to normal

Shorr In the adrenalectomized animal the kidney loses the capacity to form VEM This capacity is restored by DCA or adrenal cortical extracts

Shannon Is there any possibility of hyperreactivity in these antidiuretic states?

Shorr Dr Lee has observed a curious hyperreactivity to epinephrine in the conjunctival vessels of patients with cirrhosis There is no hyperplasia and the vessels are dilated

Shannon Hyperreactivity?

Shorr The eye vessels of cirrhotic patients were hyperreactive Our studies in animals with nutritional cirrhosis were confined to the mesenteric vessels, these evidenced hyporeactivity which is what one might expect to find in view of the predominance of VDM in the blood stream

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SOME PITUITARY AND ADRENAL INFLUENCES ON RENAL FUNCTION

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My first remarks will be on work that we have been doing on some endocrine influences on renal circulation (PAH and inulin clearances) and on PAH Tm, and more recently on the ability of the kidney to form ammonia in acidosis We know that hypophysectomy lowers these clearances and PAH Tm, we believe it does thus through loss of growth hormone Pure growth hormone, as pure as has been prepared so far, will restore the low values in hypophysectomized animals to normal and will raise the values in normal dogs to well above normal(1)

We are convinced that these great falls in clearances and Tm on hypophysectomy are not due to the following factors a) loss of thyrotropic hormone, because the drops are much greater than are seen on thyroidectomy, b) loss of gonadotropic hormone, because no fall occurs on castration, and c) loss of ACTH, because ACTH given to the hypophysectomized animal does not have any effect in restoring the low levels to the normal, which growth hormone does

Our best growth hormone preparations (Armour s 22KR2 and a preparation made in the Biochemistry Department at our school by Dr Krahl, Dr Park, and Dr Daughaday) have been almost but not completely homogeneous electrophoretically On bioassay, the best preparations have been almost free from other hormones The enhancing effects on renal functions produced by the growth hormone cannot be ascribed to the thyrotropin content, because if we give a normal dog a dose of "pure" thyrotropin eight to ten times as large as the amount of thyrotropin contained in the doses of growth hormone that we are giving we get rises of a few percent, whereas the growth preparations cause up to 100 percent increase

We have further evidence that the fall in these clearances and Tm on hypophysectomy is not due to lack of ACTH, in that not only is ACTH ineffective in restoring these depressed values of the hypophysectomized animals but neither is DCA nor whole

adrenal extract in amounts adequate to keep the adrenalectomized dog's kidney function normal nor a combination of the two We therefore feel that the most reasonable viewpoint at present is that these falls in renal circulation and in Tm are due to lack of growth hormone or to something not yet separated from it

The situation became less clear however when we found that the second (3PKS3R) and third (H1902) Armour batches did not work on normal dogs although they did on hypophysectomized A dose of 0 5 mg per kg per day of the first batch (3PKR3) was effective but when amounts of the second and third batches equivalent to 0 5 mg of the first batch were given no effects were obtained on normal dogs although the hypophysectomized dogs still responded(2) Dr I M Bunding of the Armour Laboratories has been very kind in giving us several batches of growth hormone and in supplying data on the bioassays for contaminant hormones

Table III shows that when 0 5 mg per kg per day of Armour's first batch (3PKR3) is given subcutaneously to a normal dog there is no effect on the clearances by the fifth day but a striking effect by the twelfth day Filtration fraction practically always falls The lower part of Table III shows the depressant effect of hypophysectomy on the clearances and Tm and the restoration with growth hormone The effect comes on sooner than with the normal dog and we will see later that the hypophysectomized dog will respond to relatively inactive preparations which have no effect on normals

Table IV shows the effects of growth hormone (3PKR3 active on normal and hypophysectomized dogs) and of growth hormone plus lipoadrenal extract (Upjohn) on the clearances and Tm of a DCA supported adrenalectomized dog The DCA support was adequate in that PAH Tm was essentially normal and clearances and plasma sodium and potassium levels were normal although plasma NPN was somewhat elevated I may say that we have never been able on DCA alone to keep the NPN of adrenalectomized dogs down completely to normal it may not be much above normal but is always slightly elevated even though the inulin clearance may be quite normal

Thorn How much DCA did you give the DCA supported animal?

White Three pellets of 75 mg each That was on January 31 1948 and the dog lived for eighteen months with no subsequent

TABLE III*

EFFECTS OF GROWTH HORMONE ON RENAL FUNCTIONS IN NORMAL AND HYPOPHYSECTOMIZED DOGS†

	PAH clearance	Inulin clearance	PAH Tm	Plasma Glucose
NORMAL DOG K43	ml/min/M^2	ml/min/M^2	mg/min/M^2	mg %
10/28/48 Normal	352	116	20	81
10/29/48 through 11/10/48 – 0 5 mg/kg growth hormone (3PKR3)				
11/3/48 5 days of growth hormone	336	117	27	81
11/10/48 12 days of growth hormone	849	170	33	91
11/12/48	608	138	20	79
11/16/48	308	91	16	80
HYPOPHYSECTOMIZED K39				
6/13/47 Normal	264	95	19	
6/16/47 Normal	263	96	23	
6/20/47 Simple hypophysectomy				
7/17/47	158	63	61	
9/10/47	176	44	88	
12/31/47	151	45	86	66
2/16/48	129	52	56	71
11/22/48	124	51	68	65
11/24/48 through 12/3/48 – 0 5 mg/kg growth hormone (3PKR3)				
11/29/48 5 days of growth hormone	235	82	17	106
12/3/48 9 days of growth hormone	261	95	18	99

* Tables III & IV reprinted from Am J Physiol 157 47 (1949)
† Growth hormone given daily subcutaneously

TABLE IV

EFFECTS OF GROWTH HORMONE ON RENAL FUNCTION IN AN *ADRENALECTOMIZED* DOG*

K41	PAH clearance	Inulin clearance	PAH Tm	Plasma NPN	Plasma Glucose
	ml /min / M^2	ml /min / M^2	mg / min /M^2	mg %	mg %
1/21/48 Left adrenalectomy, 3 pellets (75 mg each) *desoxycorticosterone* acetate implanted					
1/28/48 Right adrenalectomy					
2/24/48	213	86	12	37	75
10/25/48	276	96	14	46	85
10/27/48 through 11/8/48 – 0 5 mg /kg growth hormone (3PKR3)					
11/1/48 5 days of growth hormone	313	110	17		115
11/8/48 12 days of growth hormone	265	76	16	48	91
11/12/48 through 11/24/48 – 0 5 mg /kg growth hormone (3PKR3) and 0 2 ml lipo adrenal cortex					
11/18/48 5 days of hormones	229	89	6 0		74
11/24/48 12 days of hormones	182	96	4 8	57	78

* Growth hormone or growth hormone plus lipoadrenal cortex given daily

TABLE V

EFFECTS OF RELATIVELY INACTIVE GROWTH HORMONE PREPARATIONS ON RENAL FUNCTION IN NORMAL DOGS

Date	Remarks	PAH ml/min/M²	Inulin ml/min/M²	C O L/min/M²	O_2 ml/min/M²
	DOG K44 NORMAL				
4/11 & 4/12	Control	246	91	3.85	121
4/29	After 9 days of 1.25 mg/kg/day 3PKS3R	260	91		
5/5	6 more days of 2.5 mg/kg/day 3PKS3R	188	92		
5/16	10 more days of 0.5 mg/kg/day H1902	206	83		
	Interval of 3 weeks without treatment				
6/16	After 7 days of 1.25 mg/kg/day 3PKS3R	178	101		100
	DOG K46 NORMAL				
5/19 & 5/20	Control	222	94	3.46	123
5/31	After 10 days of 0.7 mg/kg/day H1902	236	121		
6/6	6 more days of 1.4 mg/kg/day H1902	192	84		
6/7	1 more day of 1.4 mg/kg/day H1902				116

therapy, finally dying *of adrenal insufficiency* The growth hormone experiments were nine to ten months after pellet implantation We cannot say that there is *no response, but it is not comparable* to that of normal or hypophysectomized dogs

Table V shows that our second and third batches in doses containing amounts of growth hormone equal to or double the amount *in 0 5 mg of 3PKR3 had no effect on clearances of normal dogs* except once on the inulin The following table, Table VI, shows *that these same preparations were active on hypophysectomized* dogs except for a failure with inulin in K42 on 4/18 the hypophysectomized dog is *much more sensitive than the normal*

Bradley Did the body temperature remain constant?

White Yes There is an increase in oxygen consumption We have never seen a renotropically active preparation that did not increase the oxygen consumption, this increase is not due to its thyrotropin content An amount of thyrotropin equivalent to the amount contained in these preparations gives no increase in oxygen consumption or clearances in either normal or hypophysectomized dogs While we have not given these preparations to thyroidectomized dogs we have in the past given crude anterior lobe *preparations to thyroidectomized dogs with consistent increases in* oxygen consumption This has some direct effect on tissues in *general though presumably it is not thyrotropin* and does not act through the thyroid gland

Dock Are there any effects on the kidney with this?

White Within the time that these were given ten to twelve days there has been no change in the dogs body weight These are all adult dogs We have not killed these dogs at the end of twelve days to see whether or not their kidneys were hypertrophied we would have to kill many dogs to find out I do not believe that these *doses of growth hormone for this short period* of time will produce any significant increase in kidney size We have *positive information* about that in the rat obtained by Dr Bunding in the Armour Laboratories Rats were hypophysectomized at 50 grams Two weeks later they were started on growth hormone one group receiving 12 5 and the other 50 gamma per rat per day continued for fourteen days The body weight increased between 0 9 and 1 gm per day while liver per body weight and kidney per body weight ratios remained the same as in untreated

TABLE VI

EFFECTS OF RELATIVELY INACTIVE GROWTH HORMONE PREPARATIONS ON RENAL FUNCTIONS IN HYPOPHYSECTOMIZED DOGS

Date	Remarks	PAH ml/min/M²	Inulin ml/min/M²	C O L/min/M²	O_2 ml/min/M²
	DOG K39 HYPOPHYSECTOMIZED				
4/7 & 4/8	Control	126	39	2 87	63
4/21	After 13 days of 1 25 mg/kg/day 3PKS3R	221	95		
4/22	After 14 days of 1 25 mg/kg/day 3PKS3R			2 86	100
4/25	After 17 days of 1 25 mg/kg/day 3PKS3R			2 74	104
	Interval of 6 weeks without treatment				
6/13	After 10 days of 1 mg/kg/day H1902	225	102		
6/14	After 11 days of 1 mg/kg/day H1902	187	87		145?
	DOG K42 HYPOPHYSECTOMIZED				
4/5 & 4/6	Control	140	58	2 25	78
4/18	After 11 days of 1 25 mg/kg/day 3PKS3R	195	54		
4/19	After 12 days of 1 25 mg/kg/day 3PKS3R			1 91	103

animals i e the percentage of organ weight increase on growth hormone was no greater than that of body weight increase

Taggart Might not the proximal segment have hypertrophied out of proportion to the rest of the kidney as reported with the balance of the adrenal corticoids?

White No measurements of nephron segments were made we do not know whether there was a demonstrable increase in proximal tubule size whether the machinery was larger or just turning over more rapidly I do not believe that growth hormone administration of this duration would produce anything like the percentage increase in size of even one segment comparable to that of increase in PAH Tm which may go up from a third or fourth of normal to normal

Thorn I take it the adrenalectomized animals did not show increase in oxygen consumption with this particular preparation

White Unfortunately no oxygen determinations were made on the adrenalectomized dog The renotropically active preparations always produced a rise in oxygen consumption in normal dogs and still more so in those hypophysectomized

Thorn We have shown that cortisone will cause an increase in basal metabolic rate which is not mediated by the thyroid(3)

White Some preparations have produced a rise in oxygen consumption without rises in clearances but we have never seen a rise in clearances not accompanied by a rise in oxygen consumption

Gilman Will you comment on the filtration factors?

White We not infrequently see filtration fractions higher than usually found — we had one about 0 40 Our average is about 0 30 or 0 31 As does every one else we always find it much higher than in man I find it difficult to believe that a dog ever normally filters 40 percent of the water going through the glomeruli but this is what we sometimes find on PAH and inulin clearances The extraction of diodrast — the work was done before the days of PAH — at low plasma levels is the same in the hypophysectomized as in the normal dog i e we believe that diodrast and presumably PAH clearance is just as good a measure of renal plasma flow in the hypophysectomized dog as it is in the normal We have no observations as to the behavior in the adrenalectomized animal We have also seen filtration fractions of 0 4 in normal dogs not

too infrequently It happens that these are high filtration fractions here but no higher than we sometimes see in the normal dog I am sure everybody has had that experience and probably not been quite sure what it means I admit it seems to me an awfully high percentage of filtrate

Lauson Have you considered the possibility of measuring actual size change in the kidney by intravenous pyelography? A radiologist assured me once that one could obtain at least an indication You could then control it in the same animal

Dock With straight films of bellies on dogs you can get lovely kidney shadows and if you take them at four or five feet you can make measurements with great accuracy

White Do you think you could detect a 20 percent change?

Dock A 20 percent change in length would be striking

White We have not done that I don t believe that there is a change in mass comparable with the change in clearances

We have been concerned about the ineffectiveness of certain growth hormone preparations in normal dogs One can think of three logical possibilities to explain this if one doesn t stretch the connotation of the word explain too far One possibility is that the material is not growth hormone at all that the active preparations are active because they contain some contaminant which really the renotropically active substance I use the term "renotropic to save time meaning a substance which influences kidney function without any commitment as to how or why it does In other words maybe it was not growth hormone but something that was present in the active preparations and the inactive preparations were inactive not because they did not have enough growth hormone but because they did not have this contaminant

The second and just as logical possibility is that the active preparations were active because they were reasonably pure growth hormone and the inactive were inactive because they contained an inhibitory substance which prevents exhibition of the renotropic without preventing the exhibition of growth promoting activity

The third possibility is that the mother molecule of the growth hormone has both growth promoting and renotropic activity but in preparation some batches suffer some damage in such a way that the renotropic activity is impaired without the growth promoting activity being correspondingly impaired

These are three possibilities one just as good as the other from the standpoint of logic but with no evidence so far to permit a choice among them

Thorn Were these different animals in the second assays or the same animals?

White Some were the same and some were different We spent considerable time trying to decide between these three possibilities we are not sure which it is I think it is the last and will give you briefly some of the evidence One thing that seemed worth doing was to get some intermediate fractions that occur in the preparation of the purification of growth hormone in the hope of finding one that would be renotropic but not growth promoting

The other possibility that one of these fractions contains the hypothetical inhibitor could be tested by adding fractions to active growth hormone preparation If inhibition occurred it could be argued that it is the presence of such postulated inhibitor in the inactive preparations that makes them inactive

I will make the story brief by saying that neither such activity has been found Dr Wilhelmi was kind enough to send us some of the fractions that he had obtained in various preparations that he made He sent us some of his fractions C D and E and one which he calls PH5dep those who are acquainted with the process will recognize these All we can say is that none of these substances when given to an animal had much effect on clearances In other words there was no evidence that any one of these fractions investigated contained the postulated renotropic contaminant in any concentrated form Of course if we tried five more fractions we might find one that did The other attempt to see if adding any of these fractions to an active preparation would make it inactive also has failed to produce evidence of inhibition although we have not tried all of them yet This work is time consuming and I think we have spent about as much time on it as is justified

Table VII illustrates what I mean It shows a normal dog that we tried out to be sure that she did respond to a known renotropically active preparation 22KR2 Armour's purest growth hormone preparation which is almost homogeneous electrophoretically That produced the expected effects on clearances and as you can see the filtration fraction fell The animal was permitted to recover and six weeks later was given 0 5 mg per kilogram per day of Wilhelmi's fraction D This had perhaps some

effect though not much on the PAH. That is certainly significant on the inulin. I won't say that none of these fractions did anything to the clearances but the point I am trying to make is that if they consisted largely of this hypothetical active contaminant they should have been even more active than the presumably pure growth hormone preparation was, but none of them were anywhere near as active. The animal was then given thyrotropin in amount eight times as much as is contained in our dose of growth hormone and its effect on the clearances is not comparable to that of the growth hormone.

TABLE VII

EFFECTS OF AN ACTIVE PREPARATION, OF A RELATIVELY INACTIVE FRACTION, AND OF THYROTROPIN

Date	K48 NORMAL			
	PAH	Inulin	FF	O
10/5/49	243	80	0.32	122
10/18 through 10/31 0.42 mg./kg./day G.H. 22KR2				
10/28	482	132	0.27	168
10/31	454	125	0.28	156
11/14	317	104	0.33	118
11/21	237	85	0.36	110
12/6 through 12/15 0.5 mg./kg./day Wilhelmi fraction D				
12/15	293	112	0.38	114
12/30	229	76	0.33	112
12/31/49 through 1/9/50 0.1 mg./kg./day thyrotropin PRR3				
1/9/50	280	99	0.35	116
1/19/50	278	88	0.32	

Janeway: It is interesting that it does not increase the oxygen consumption.

White: There is no increase in oxygen consumption in normal dogs with this dose of thyrotropin which is still a small one. This is merely to confirm that the thyrotropin content of this preparation 22KR2 is exceedingly low; its renotropic action cannot be explained by thyrotropin contamination.

Table VIII shows results with another fraction Here is another normal dog who responded to 22KR2 in the expected fashion Wilhelmi s fraction E had a possibly significant effect on PAH clearance and it apparently had a considerable effect on inulin clearance here We are suspicious of this inulin value however because it was done at unusually low plasma levels and because the same preparation had no effect on either PAH or inulin clear ance in another dog who responded well to 22KR2 In other words the effect if any is much less than that of the pure growth hormone preparation In considering the effects of all these fractions in several dogs we obtained no evidence for either the active con taminant or the inhibitor theory We have had two or three more extracts that were prepared in the Biochemistry Department at our school and while it does not settle the question all we can say is that no evidence has been obtained in favor of either of these first two possibilities By exclusion this would perhaps favor the view of some change in the protein molecule which influenced one activity more than it did the other

TABLE VIII

EFFECTS OF AN ACTIVE PREPARATION AND OF FRACTION E

K49 NORMAL				
Date	PAH	Inul n	FF	O
11/8/49	216	97	0 45	
11/12 through 12/2/49 0 42 mg /kg /day G H 22KR2				
11/21	434	163	0 38	181
11/23	507	151	0 30	192
12/2	381	130	0 34	
12/20	236	95	0 40	118
12/20 through 12/29 0 5 mg /kg /day Wilhelmi s fraction E				
12/29	270	142	0 52	108
1/1/50 Sick not eating died on 1/8/50 cause undetermined				

van Dyke You did not try to alter the original extract which had this effect?

White No We have thought about trying either gentle oxidation or reduction to see if we could artificially damage one property without the other

van Dyke Could you fractionate it at all? Could you separate it into your active and less active fractions and test those?

White In what way?

van Dyke Perhaps by the alcohol fractionation technique

White These fractions have come out of the Wilhelmi Fishman Russell procedure(4) and are less homogeneous electrophoretically than the 22KR2 as well as being much less active in promoting growth I think it would be quite a job if you really tried to fractionate them we have not tried to do so

van Dyke I thought that the original Armour preparation with which you had your first difficulty was less potent

White The first one was potent Fortunately we obtained a good one the first time

van Dyke But the second one?

White That was not

van Dyke That was inactive in normal dogs?

White That is right

van Dyke If you fractionated that extract might you not possibly find the source of the discrepancy?

White If we knew enough about how to fractionate it possibly we could It was prepared by a procedure identical with that used for the first preparation

van Dyke Perhaps this could be done if an adequate supply of extract were available

White None of it is left That is the trouble We get the extract in samples of about 100 to 200 mg at a time

Obviously the best way to show whether the renotropic principle is growth hormone or not is to get a preparation which you can be sure does not have anything but growth hormone in it and see if that works

I would like to discuss briefly one other point Our 22KR2 which was a very active preparation was sent to us in three bottles The

first bottle was used while it was still quite active The second was quite active, but after having been opened about two months it became less active and at the end of six months it had no activity at all in the normal dog, although in the hypophysectomized dog it still acted In other words, it spontaneously became the same material as H1902 and 3PKS3R We did nothing to it—it had been in the desiccator all the time—and I don't know why it went bad We assayed it for growth promoting activity, as seen in Figure 11

We used eleven rats, hypophysectomized at 115 gm I want to point out that these are not the conventional conditions for growth hormone bioassays Most people use 60 gm rats and start the hormone administration ten days after operation We happened to have some 115 gm rats and they were not used until six weeks after the operation So these cannot be quantitatively compared with the responses of rats of 60 gm begun ten days after the operation But the magnitude of the weight increase is about what one would expect to find with these same doses of growth hormone preparations Four rats received 5 gamma four received 10 and three of them received 20 and the average increase was about 0 9 to 1 gm per rat per day, the 20 gamma rats increasing a little more than the other two This shows that growth promoting

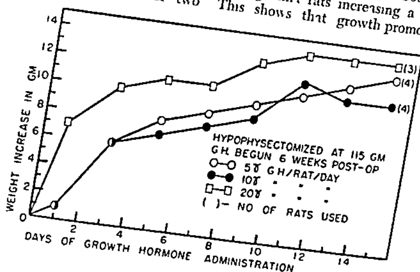


FIGURE 11

Growth promoting activity of a sample of Armour's 22KR2 growth hormone originally highly active renotropically which on standing had lost its renotropic activity in normal dogs

activity had not been greatly impaired after renotropic activity in normal dogs had been lost although it does not prove that there had been no impairment of growth promoting activity Dr Bunding took the few remaining milligrams of this sample and has subsequently found that its growth promoting activity is about 60 percent of the original so the decline in renotropic activity has been much greater than that in growth promoting activity

Thorn I want to be clear on one point The material that was active in the hypophysectomized animal and not in the normal when given two or three times the dose still had no effect?

White That is right We went up to two and one half times ie normal dose and it still did not work in normal dogs The third ottle which was opened after we found the second bottle had ne bad was just as active as the first one The only difference handling it and the second bottle was that the top had not been en off Yet it was not hermetically sealed So I cannot explain it was the matter it may have been a matter of moisture

one animal the response fell off with repeated administrations e same active preparation We know that it was not because preparation was becoming less active because at the time of the last administration to this normal dog which failed to respo it was giving good responses in other normal dogs That is t only normal dog who has acquired a tolerance to this material other dogs where repeated courses have been given they hav continued to respond except in the case where the material wen bad

Thorn Is the antibody titer to the material higher in the refractory dog?

White We have no observations on it

The arterial pressure is not changed in these animals so obviously the renal vascular resistance is decreased by what mechanism we dont know We have no observations on what would happen if the material were given to an animal with denervated kidneys although I believe that we would still get increases

Selkurt The hematocrit stays normal? Does the cell volume go down or anything of that sort?

White We did not do careful hematocrit determinations but I am sure they did not change significantly Just looking at the tubes

as they were centrifuged I know there was no significant rise or fall in hematocrit We had no reason to think there would be any change and did not measure it We leave the matter then by saying *that these preparations* do raise the clearances and the Tm in normal and in hypophysectomized dogs that these effects are not due to thyrotropin content that the hypophysectomized dog is more sensitive than the normal and that the failure of some preparations to show the effect in normal dogs may be due to the fact that there has been differential damage done to the molecule We offer that simply because we do not have any other explanation No evidence was obtained for the inhibitor theory or the active contaminant theory although these possibilities are not excluded It may be that the active material is not really growth hormone but some hitherto undescribed substance which is present in active form in some of these growth hormone preparations and not in others and which has essentially the same electrophoretic mobility as the growth hormone

I would like to say just a few words about another type of work *in which we have been interested We felt that some other kidney* functions which are susceptible to quantitative evaluation in addition to the clearances and Tm might be worth investigating as to their being influenced by endocrine factors So we produced acidosis in normal dogs to stimulate ammonia formation What we wanted to do was to establish conditions which would give us a reproducible ammonia response to acidosis and see whether the hypophysectomized and the adrenalectomized dog responded in the same way We found that when 3 5 to 5 mEq ammonium chloride per kilogram are given by stomach tube or subcutaneously to normal dogs that the plasma CO falls usually to 16 or 17 mEq within one or two hours The ammonia production rises at the same rate i e rate of ammonia production reaches its maximum and levels off within about two hours With somewhat larger doses i e 7 to 8 mEq per kilogram the plasma CO drops to 12 to 14 mEq and there is usually a greater ammonia output than with the lesser dose On two occasions we have given 10 mEq per kilogram and seen no greater ammonia output than with the 8 mEq which we gave as a standard dose In such acidosis i e in response to 7 or 8 mEq ammonium chloride per kilogram with plasma CO dropping from the normal of 23 or 24 down to perhaps 13 14 or 15 the ammonia output rises from a control value of about 0 5 to 1 5 mEq per hour to 4 5 to 5 5 mEq per hour for 12 to 15 kg dogs That will happen within two hours This happens to be about

the same per unit body weight as man's maximum ammonia output, which has been described as in the neighborhood of 20 mEq per hour(5,6) I believe Dr Pitts found about 5 5 to 6 mEq per hour as the highest outputs in dogs If this is not ammonia Tm, it is close to it Even if this is not Tm, we have established a predictable response of the normal dog to this particular dose of acid, reproducible within a reasonably narrow range With the smaller doses, e g , 3 5 to 5 mEq per kilogram, the plasma CO_2 has already begun to rise and the rate of ammonia excretion begun to fall at the end of three or four hours after the acid administration With the larger doses, up to 7 or 8 mEq per kilogram, the plasma CO_2 is still down four hours later and the ammonia output has not yet begun to fall By the next morning, however, provided the doses are not larger than 8 mEq per kilogram — and we have not investigated larger doses from this standpoint — twenty-four hours after the acid is given, the CO_2 level and ammonia output are back to normal in normal dogs, in that respect they differ quite markedly from the human subject If we now compare a hypophysectomized dog with the normal we find that he behaves the same as the normal The number of experiments on this is not as large as it should be but so far our finding is that the ability of the kidney to form ammonia is unchanged in the first few weeks after hypophysectomy

This finding contrasts with that in a single adrenalectomized dog with inadequate DCA support This dog had been adrenalectomized a year and one-half before these experiments and up until about a month before the experiment had remained in excellent condition with the 225 mg DCA subcutaneously She began then to show definite signs of adrenal failure, loss of appetite, weakness, and beginning fall in plasma sodium which had been between 140 and 145 all this time but now gradually went down It fell as low as 130, with some rise in potassium During this time the animal was going down hill, and was in marked adrenal insufficiency when the ability to form ammonia was tested Of course, the animal had acidosis even before she was given the ammonium chloride the plasma CO_2 was running around 19 compared to a normal of 23 or 24 With relatively small acid administration (2 2 to 3 3 mEq per kg), plasma CO_2 fell to 11-13 mEq , but there was no increase in ammonia production This was not because the ammonia production was already high The control outputs, without giving acid, were 0.2 to 1 3 mEq per hour, the same as with normal animals on a comparable diet This animal was kept on a low meat and high milk diet So it appears then that the kidney

in an inadequately supported adrenalectomized dog shows great impairment of ability to form ammonia in response to acute acidosis Most of you have seen in the *September 1950* issue of the *American Journal of Medicine* that Dr Pitts reported somewhat similar findings on adrenalectomized rats and found at least a partial restoration of ammonia forming ability on DCA or cortisone(7)

In Figure 12 is plotted the highest ammon a excretion in mEq per kg per hour against the lowest plasma CO_2 in a given experiment Usually the lowest plasma CO coincided with the period of highest ammonia never more than an hour s difference most of the plotted points being in the third or fourth hour after the acid dose When the normal animal has the plasma CO lowered to 14 to 17 mEq the ammonia output is in the neighborhood of 0 4 mEq per kilogram per hour With still greater acidosis where plasma CO levels are below 13 rate of ammonia output is usually between 4 and 5 per kilogram per hour The hypophysectomized dog — one animal — shows a response not significantly different

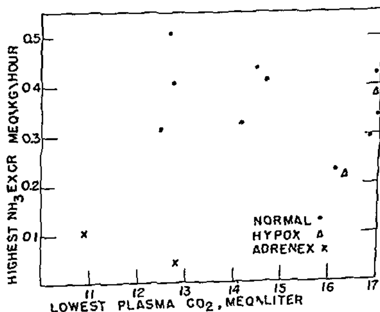


FIGURE 12

f mmon ou put plotted ag in t lo est plasma CO levels n ... dog ... ncy

from the normal, whereas in two observations on the adrenalectomized dog, even though acidosis was more severe than it had been in the normal or the hypophysectomized, there is essentially no rise in ammonia output We have no observations on restoration with adrenal cortical therapy, the only observation being on this dog which is already pretty well down hill

We have known for quite a while that the hypophysectomized dog on a normal diet handles electrolytes in a quite normal fashion, maintains normal plasma sodium and potassium, stays in electrolyte and water balance, and does not develop edema or salt depletion It seems then that the formation of the salt-regulating hormone is not under anterior lobe control We also know that the *zona glomerulosa* of the dog does not atrophy after hypophysectomy, whatever that may be worth in concluding anything as to the site of formation of the DCA like hormones as compared with the others That is also true in the rat We also know that ACTH in human subjects usually produces some salt retention, at least as the initial effect This could mean that there is an overlapping of function, that the hormones not primarily concerned with salt retention have some salt-retaining effect, or it could mean that the *zona glomerulosa*, if that is the place where DCA-like substances are formed, is a little bit responsive to ACTH It seemed to us that the obvious thing to do, in order to see whether the ability of the hypophysectomized dogs' adrenal cortex to form salt regulating hormones was normal or not, was to put it under stress In other words, the fact that he maintains normal plasma sodium and stays normal when he is on a normal salt intake is not a very severe test So we put such dogs on a very low sodium intake, 1 mEq a day This was done by giving Lonalac, a sodium free milk powder, as the source of protein, plus fat and carbohydrate and added vitamins On such a regimen the hypophysectomized dog does go into sodium deficit a very extensive negative sodium balance The plasma sodium dropped to 123 where it had been 143, and the dog would have been dead in another day, I am sure, if we had not given her a large dose of salt and resumed a normal diet In one case it took only two weeks for this to come on, in another case it took six weeks It appears that the adrenal cortex of the hypophysectomized dog is not completely normal so far as its ability to form DCA-like hormones is concerned, but it is autonomous enough to get along quite well, so far as salt regulating hormone is concerned as long as the animal is on a normal, not necessarily high, salt intake He cannot stand severe salt depriva-

tion indefinitely If at this point we give these dogs a large dose of salt, they retain it We gave them 130 mEq and they did not put any out in the next twenty four hours, not more than 2 or 3 mEq, *they soak* it up It seems as though they hold more salt than you would think they had room for as calculated on the basis of our rather inadequate calculations of body fluids

Selkurt Does the hypophysectomized dog without preliminary salt deprivation have a tendency to hold on to salt or lose it when loaded?

White He has a perfectly normal salt test, i e, output response to loading

Selkurt Only when you initially deprive them does that phenomenon come on?

White We give the normal dog a big dose of salt, 60, 70, 80 to 100 mEq in the form of 0 9 percent sodium chloride by stomach tube and follow the rate at which that comes out in the urine The normal dog gets rid of it in about eighteen to twenty four hours while it takes a man about four days It follows a fairly reproducible curve, the maximum output being in either the second or the third three hour period all or most of it is out in eighteen hours and all is out in twenty four The hypophysectomized animal behaves in the same way as the normal, there is no evidence that he loses it any faster or any more slowly than the normal Also, when the normal dog is put on this very restricted salt diet, he gets along perfectly well He just reduces his output to the level of his intake if there is any negative balance it is very small The normal dog we can say can stand at least eight weeks of this very restricted salt intake whereas the hypophysectomized dog stands it from two to six weeks

Shorr You might consider another type of experiment, that is, raising the metabolic rate with thyroid Occasionally, when we attempt to restore the basal rate of a patient with myxedema by giving thyroid, we find that the myxedema is of pituitary origin As a result of thyroid therapy the relative adrenal insufficiency will now become apparent and the patient will go into an adrenal crisis

White Yes

Thorn You cannot raise the BMR of the hypophysectomized animals with thyroid?

Shorr No, you cannot

Thorn It is well known that moderate doses of thyroid are ineffective in correcting the hypometabolism of pituitary-deficient patients

White I cannot speak from experience about the giving of thyroid to the hypophysectomized dog but they seem to be even more sensitive than the normal dog to thyrotropin

Thorn But the metabolic response is very poor to administered thyroid hormone

White We have no experience on that I do know that they will respond to thyrotropin We have also found that inulin and PAH clearances and PAH Tm are increased by oral thyroid administration in the hypophysectomized dog even more than in the normal

It appears then that the adrenal cortex of the hypophysectomized dog is not impaired in its function of maintaining the ability of the kidney to form ammonia but that it is somewhat impaired in its function of maintaining the kidney s ability to conserve salt, although it has to be put to great stress before we can demonstrate such impairment

Dr Pitts asked me first if I would say something about endocrine influences on intracellular tubular processes I told him I did not know enough about it to discuss the matter with any authority or confidence

I might however point out one or two speculations It is possible that in the absence of the adrenal cortex the kidney s ability to hydrolyze glutamine into ammonia and glutamic acid is impaired Some of you may remember that Jiminez Diaz(8) reported decreased ammonia formation *in vitro* by kidney tissue of adrenalectomized cats in a d l alanine medium indicating lowered amino oxidase activity or concentration So far as I know that is the only observation on the change in the enzyme content or activity of kidney tissue as influenced by some endocrine factor I don t know that this has ever been confirmed or denied and I do not know of any observations on the glutaminase content of the kidney of the adrenalectomized animal as compared with the normal It may *well be that this is the reason why it does not work so well* Again I know of no observations regarding any change in the ammonia precursors in the blood in any endocrine condition I don t know of any reason to think that there is any deficit of glutamine sup-

plied to the kidney in adrenal insufficiency One might, of course, postulate that if in response to acidosis there is an increased stimulus to adrenal cortical activity, that as the result of the neoglucogenesis, protein breakdown, there might be an increased supply of amino acids available as precursors for ammonia production That seems a little farfetched to me, I just don't know We know that Lotspeich and Pitts(9) showed some time ago that if you do give the animal more amino acids when he is in acidosis you can increase his ammonia production, and it is conceivable that some such mechanism exists It has also been known since the time of Walter back in 1877 that the rabbit is much less capable of forming ammonia in response to acidosis than is the dog or man(10), and again one might speculate that perhaps the rabbit's kidney does not have enough glutaminase or renal amino oxidase Perhaps someone here knows some published work which I have missed, or some work in progress and not yet published, on the enzyme content, either glutaminase or amino oxidase, of the kidneys of hypophysectomized or adrenalectomized animals as compared with the normal We would assume from these findings, if they turn out to be consistently true, that the hypophysectomized animal's kidney enzyme system is all right as far as ammonia production is concerned, but not the adrenalectomized

DISCUSSION

Gaunt I would like to ask two questions of Dr White One concerns your work on how these hypophysectomized animals handle salt loads Apparently you do not agree with Dr de Bodo(11) since you found that sensitivity to salt restriction took a long time to develop, did you not? Second, how do the hypophysectomized animals handle water?

White We have been fortunate in this work in some ways The first time, the very first growth hormone preparation we tried worked well If it had not we probably would have quit then The very first hypophysectomized dog we put on 1 mEq of sodium a day went into severe salt deficiency in two weeks The next one took six weeks If it had been the first one we probably would have stopped after about four weeks I should have refreshed my memory as to how long de Bodo followed these dogs on restricted salt intake

I might say this, we had one hypophysectomized dog which never did go into salt deficiency, and about the time we were becoming concerned about that she came into heat and we found at autopsy that there was some anterior lobe left That is, of course, always a possibility Any conclusion that the anterior lobe was not necessary must be held in abeyance until the autopsy shows complete absence of anterior lobe We have repeatedly seen small fragments of anterior lobe regenerate I don't mean we have watched it We have seen females that would come into heat after two years This may sound fantastic, but one of those dogs was bred and did not become pregnant, six months later she came in again and was bred and did become pregnant but miscarried, six months later she was bred again and carried her pups to term but could not nurse them six months later she had pups at term and nursed them normally We interpret that as a small bit of anterior lobe growing larger Just such isolated observations as that show that it can and does happen

Shock Do you have any ideas about the mechanism whereby the Tm can be increased in normal animals?

White I don't know except that it must have something to do with enzymatic processes, which is another way of saying I don't know It makes the wheels go around faster I think rather than making them bigger although this is only a guess

Taggart We have a limited amount of information which may have some bearing on this point Last year I described our technique for studying PAH transport in kidney slices Since then we have treated a number of rabbits with either testosterone or thyroxin for six days before they were sacrificed for the preparation of kidney slices The doses administered were essentially the same as those which have previously been found to produce significant increases in diodrast Tm in dogs(11,12) Thyroxin administration increased the respiration of kidney slices to approximately 30 percent above the normal controls, whereas testosterone had no appreciable effect on respiration Neither agent significantly altered the rate of PAH transport Certainly in the case of thyroxin, the machinery of oxidative metabolism was turning over faster than normally, but this was not reflected in the rate at which PAH was transported in the slice

White Had those doses increased the Tm in the living rabbit?

Taggart We do not know

White You did not determine that? I don t remember about the rabbit I remember that testosterone is said to raise it in the dog(11) but not in man(13)

Taggart The point which I wished to emphasize was simply that a speeding up of the metabolic machinery as observed with thyroxin apparently does not increase the transport capacity

White The enzymologists point out instances where the oxygen consumption is not impaired and still the transfer mechanism is impaired

Taggart A good example is dinitrophenol which can increase the respiration of kidney slices up to 100 percent above normal but at the same time markedly depresses PAH transport

White An instance where an agent is known to produce increased transfer in the living animal but not increased transfer per unit weight of kidney *in vitro* would favor of course the view which you no doubt hold that maybe this is merely an increase in mass of tubular tissue and not a faster turnover

Taggart I was wondering whether the effects which you have observed with growth hormone were associated with any morphological alteration of the nephron

White We have no observations on that It might be possible to detect whether there is a change in kidney size through x ray observations as Dr Dock suggests I did not realize you could do that

Taggart An overall measurement of kidney size would not be entirely satisfactory I am more interested in knowing what happens to the various portions of the nephron

Grafflin Acromegaly as is well known from the work of Cushing and Davidoff(14) and others is usually characterized by hepatomegaly and nephromegaly Cushing and Davidoff reported that the enlarged kidneys (and liver) were histologically well within normal limits and I believe this has been the general experience of interested pathologists I should like to call attention to the work of Turley who around 1916 made an entirely unique wax reconstruction of the glomerulus and proximal convoluted tubule in acromegaly(15) In this case the weight of the kidneys was approximately twice the normal and no abnormal histologic change was observed The essential facts contributed by the model are

that the *pars convoluta* of the proximal tubule of the particular nephron reconstructed had undergone a striking increase in length but not in diameter, and that the increase in length was roughly proportional to the enlargement of the kidney as a whole (approximately twice normal) The size of the glomerulus was within the normal range ,

Taggart Selye has described the effect of testosterone administration in mice(16) The kidneys of treated animals increased their weight by 50 percent or more but essentially all of the increase was attributable to hypertrophy of the proximal and distal convoluted tubules

Thorn To check the growth hormone work on the dog you would like to know the Tm is very high in the acromegalic would you not? Has not anybody ever reported one?

Grafflin I dont know of one

Selkurt May I ask a question in regard to the change in plasma flow relative to Tm? Does the Tm increase to a greater degree than the effective plasma flow or does it go up proportionately? It might throw some light on whether this is real growth or whether the kinetic mechanism is speeded up You would expect that the vascular channels would grow in proportion if the effect is due to hypertrophy

White The Tm in the hypophysectomized dog is lowered more than the plasma flow is and they are both raised essentially to normal by growth hormone so that the Tm is raised more than the PAH clearances in the hypophysectomized animal In the normal dog the Tm is usually not raised quite as much as the clearance as we frequently double the PAH clearance in the normal dog and usually somewhat less than double the Tm In the hypophysectomized dog we again double the clearance and triple the Tm it has been down to a third of normal and is raised to about normal

Shannon One thought occurs to me that might be pertinent to this discussion We are considering whether increased ability to transport PAH is representative of hypertrophy or representative of increased activity Possibly Dr Taggart might comment on his experience with the simple administration of acetate Acetate increases transport rapidly and about as strikingly as Dr White s growth hormone

Taggart Naturally I am interested in the possibility of differentiating the effect of acetate, which occurs within ten to fifteen minutes, from that of the hormones which I believe develops only over the course of several days

White In normals we usually don t see anything in the first five days It will be seen in ten days but usually not in five In the hypophysectomized the increase will be seen in five days

Shannon How rapidly does it decline Dr White?

White After five days of administration the PAH clearance was 336 ml per minute with a normal of 352 ml per minute thus no change was observed In twelve days it was doubled or more Two days after we stopped it was well on its way down and six days after we stopped it was below

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THE ACTIONS OF ACTH AND CORTISONE ON RENAL FUNCTION IN MAN*

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OUR INQUIRY into the effects of ACTH and cortisone was conducted along two general lines(1,2,3) (A) The effects of large doses of *these substances on standard renal clearances and on the simul* taneous urinary excretions and plasma levels of a number of the constituents of the urine have been determined in normal human *subjects, in patients with Addison's disease, and in isolated patients* with glomerulonephritis and with periarteritis nodosa (B) We have attempted to quantitate certain aspects of the abnormalities *of water and salt metabolism in Addison s disease before* and after cortisone therapy These latter investigations were directed espe cially toward studying the effect of this steroid on the inability of *patients with adrenal insufficiency to excrete* excessive quantities of sodium and water

Special mention should be made of the dosages which were employed in these studies Really very large doses – up *to 400 to* 600 mg of ACTH a day and comparable quantities of cortisone – were given to the normal subjects The reason for this was that in *these normal individuals it was found that doses of this magnitude* were required in order to demonstrate an ACTH or cortisone effect in terms of increased uric acid excretion eosinopenia and weight *gain appreciable effects on renal function were* obtained also only with the large doses

With ACTH, C_{In} and C_{PAH} were both significantly increased as shown in Figure [illegible] one instance and was unchang[illegible]ses of 100 to 200 mg a day [illegible] and C_{PAH} *similarly changed in the one instance where measured By* increas ing the dose to very large quantities C_{In} now was significantly

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* [illegible]

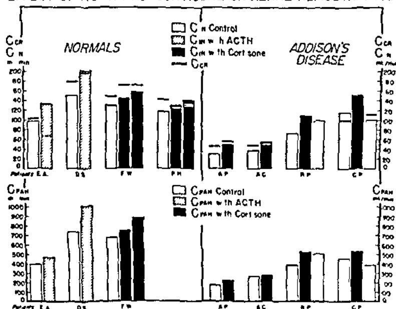


FIGURE 13

increased in both instances and C_{PAH} in the one instance where measured. The filtration fraction did not change. In the group of patients with Addison's disease control filtration rates varied from very low to normal values. In each instance a significant increase in C_{IN} was effected by the administration of cortisone. The doses in these patients were not as large as in the normals — 100 to 200 mg a day — but as has been pointed out for an Addisonian this is far above physiologic requirement for maintenance. C_{PAH} also increased slightly but here the change was not significant, therefore among the Addisonians filtration fraction rose.

In the entire group but more particularly in the normal group although plasma flow was increased the calculated blood flow was not significantly altered. The reason for this was that under the condition of the experiments hematocrits uniformly fell in about the proportion that C_{PAH} clearance was increased. Tm_{PAH} was unaffected by ACTH and irregularly changed with cortisone, rising in some instances and falling in others.

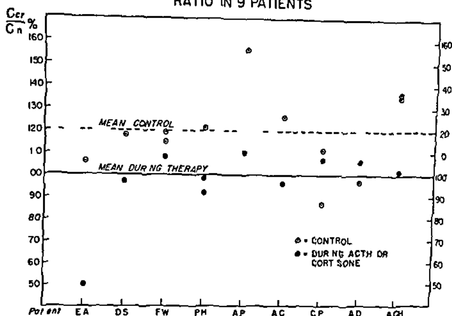


FIGURE 14

Simultaneous creatinine and inulin clearances were measured in nine patients (Figure 14) During the control studies average C_{cr}/C_{In} exceeded 1 0 in all groups averaging 1 2 During therapy there was a significant drop with an average now for the entire group of 1 0 Examination of changes in these ratios in individual patients demonstrates two exceptions to these average figures in one patient with Addison's disease (C P) and one with glomerulonephritis (A Ch) the ratios rose on therapy These decreases in ratio of C_{cr}/C_{In} were therefore fairly consistent and significant In only one patient (E A) who received ACTH was actual *excretion* or *clearance* of creatinine significantly changed by either agent In this patient C_{cr} fell from 100 ml per minute during control studies to 67 ml per minute during ACTH administration

No adequate explanation for these findings is apparent from the data Increase in extracellular space as inferred from the decreased hematocrit and weight gains occurred regularly Since at least in the normals blood pressure did not rise there is no evidence that glomerular hydrostatic pressure was increased Hemodilution *per se* would be expected to increase plasma flow if all the other relevant

factors remained constant and C_{PAH} was indeed increased in each of the normal subjects in approximate proportions to the changes in hematocrit If it is assumed that the amount of filtrate formed is limited by the increase in colloid osmotic pressure of the unfiltered plasma as it flows through the glomerulus then it would seem reasonable to conclude that with a greater plasma flow more filtrate would be formed per unit time before the same limiting protein concentration would be reached With increased plasma flow greater diffusion of fluid might also be expected to occur irrespective of changes in pressure relationships Thus increased plasma flow might increase filtration rate without the intervention of changes in glomerular pressure or of changes in the size and permeability of the filtering bed

In the patients with adrenal insufficiency C_{PAH} increases were slight while those in C_{In} were usually large It is more difficult therefore in this group to attribute increased glomerular filtration rate to increase glomerular plasma flow In three of these four patients increased glomerular hydrostatic pressure can be postulated because small increases in arterial blood pressure occurred following cortisone

Alterations in glomerular hydrostatic pressure might also have been produced by changes in efferent or afferent arteriolar tone and could have resulted in large changes in filtration independent of those conditioned by glomerular plasma flow or systemic arterial pressure There are no observations in these experiments which would indicate whether or not such vasomotor changes did in fact occur

If the improved C_{In} represented an increase in the total functioning renal mass similar to the renal hypertrophy produced in animals by large doses of steroids(4 5) or if it were the result of an activation of previously functionless nephrons one would expect proportional increases in Tm_{PAH} Changes in C_{In} Tm_{PAH} were not at all constant however no change increases and decreases were observed

The rise in C_{In} without proportionate increase or with no rise at all in C_{Cr} can be variously interpreted If creatinine is secreted and inulin actually measures filtration rate then one must conclude that decreased tubular secretion resulted and indeed in E A there must have been actual reabsorption of creatinine because creatinine clearance fell well below inulin clearance Alternatively if inulin is reabsorbed reduced tubular permeability to this substance must

have occurred In any event the conclusion seems inescapable that tubular transport of creatinine or inulin or both must have been altered

Excretion studies of uric acid and phosphorus further support the premise that adrenal steroids act in part at least by changing *renal tubular function* *In keeping with other reports*(6 7) urine uric acid excretion increased markedly despite lowered serum concentrations in three of our four patients and further this increased excretion was accomplished in the presence of a diminished filtered *load of uric acid*

Since it appears that uric acid is normally totally filterable at the glomerulus(8 9) and reabsorbed by the tubules(10) the data suggest that the uricosuric effects of ACTH and cortisone are the result of altered renal tubular transport Adrenal hormones may accelerate tubular secretion of uric acid if such occurs(11) or inhibit tubular reabsorption or both

Further evidence for a primarily renal mechanism for the uricosuric effect of ACTH and cortisone is furnished by the observation that the administration of adrenal cortical extract to nephrectomized animals does not alter the rate of accumulation of uric acid in the serum(12)

Similar conclusions may be drawn from phosphorus excretion The well established phosphaturia resulting from administration of these hormones was demonstrable despite diminished or constant *filtered loads of these anions* *Therefore it seems possible* that alterations in the renal tubular transport of phosphorus may well account for the losses of phosphate exceeding those of nitrogen and for those losses occurring in the absence of negative nitrogen and *calcium balance* *In one patient (E A)* who received ACTH renal tubular secretion of phosphate apparently occurred Phosphate clearance was 150 to 190 percent of the inulin clearance during two consecutive periods We have been unable to repeat this observation in subsequent patients even by combining sodium para aminohippurate and phosphate loads The data are therefore presented merely for your consideration but so far as we have been able to determine they are right At any rate it does seem likely that tubular transport of phosphorus has been altered

The data on sodium and potassium excretion in normal subjects can be quickly summarized Day to day excretion in keeping with the finding of many others showed sodium retention with both

EFFECT OF ACTH ON ELECTROLYTE EXCRETION DURING NaPAH LOAD

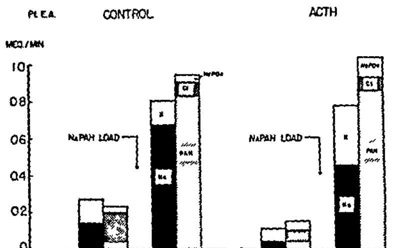


FIGURE 15

agents usually more with ACTH than with cortisone Potassium excretion was inconstantly increased or not increased at all Attempts to demonstrate changes in tubular activity relative to changes in quantities of these cations filtered and reabsorbed during acute experiments were by and large unsuccessful

Under one circumstance however a reproducible and interesting phenomenon occurred as shown in Figure 15 Whereas Tm_{PAH} determinations during control studies resulted in increased urinary

termina

demon

dium by

potassium so that the administered sodium para aminohippurate was now being excreted as potassium para aminohippurate Although there are no good explanations for these phenomena one might speculate that the effect of adrenal steroids on potassium excretion may depend upon an augmentation of sodium reabsorption and upon the nature and quantity of the anion claiming excretion Possibly related to these findings is the fact that in patients with the nephrotic syndrome administration of sodium loads results in the excretion of large amounts of potassium(13 14) Furthermore it has recently been noted that DCA does not produce increased

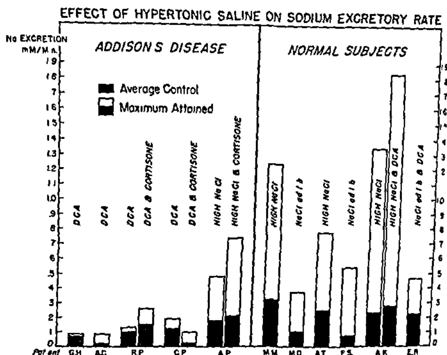


FIGURE 16

potassium excretion when given to patients receiving a low sodium diet* and in whom sodium reabsorption was already near maximal This hypothesis of the mechanism underlying the increased potassium excretion which sometimes accompanies ACTH or cortisone administration is consistent with a recent suggestion(15) that intratubular exchange of sodium and potassium may occur

The effects of hypertonic solutions of saline were studied in five patients with Addison's disease (Figure 16) As expected from previous experimental and clinical observations there was a marked difference between patients with adrenal insufficiency and normal subjects studied under similar conditions Thus in four of five patients urinary sodium excretion increased only slightly following administration of approximately 4 percent saline intravenously and there was no overlapping between these four and the normals In the fifth patient during control studies while on a previously high salt intake but no DCA the urinary excretion following hypertonic saline equalled or exceeded that of two normals The same test

* D W Seldin L C Welt and J Cort Personal communication

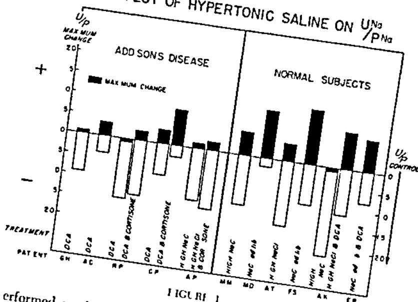


FIGURE 1

erformed in three patients after cortisone (150 to 200 mg per n for four to seven days) slightly improved excretion in two tients and definitely improved excretion in the third patient. A had no effect on the response of one normal and in another rhaps the response was conditioned and diminished by this steroid.

This inability to excrete the sodium load allows at least two gross explanations. First, urine flow did not increase; diuresis simply did not follow administration of hypertonic saline. Second, the poor sodium excretion was also explained by the relative inability of these patients to increase urinary concentration above control levels (Figure 1*). Control concentration and U/P of sodium varied rather widely in the patients with adrenal insufficiency as indicated by the blocks below the line. Ability to increase U/P* was sharply limited before cortisone as compared with the normals. The one apparent exception to this among the normals (A. K.) should be mentioned. In this instance as a result of a high previous salt intake control concentration was so high that presumably maximal urinary

* Al I n gl ar sod ... I to 8 mEq per L d r ng the experi ... sod ... concentrat on changes ... re so n ... greater that U/P changes reflect essentially changes in urinary concentration.

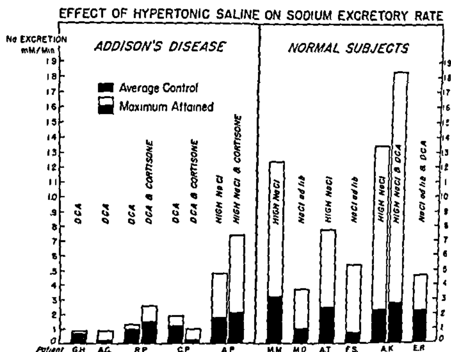


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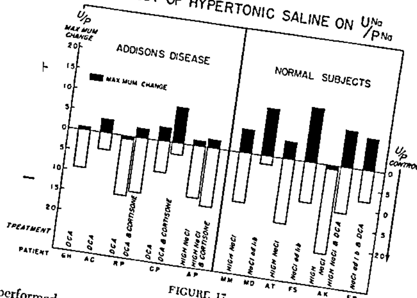


FIGURE 17

performed in three patients after cortisone (150 to 200 mg per day for four to seven days) slightly improved excretion in two patients and definitely improved excretion in the third patient DCA had no effect on the response of one normal and in another perhaps the response was conditioned and diminished by this steroid

This inability to excrete the sodium load allows at least two gross explanations First urine flow did not increase diuresis simply did not follow administration of hypertonic saline Second the poor sodium excretion was also explained by the relative inability of these patients to increase urinary concentration above control levels (Figure 17) Control concentration and U P of sodium varied rather widely in the patients with adrenal insufficiency as indicated by the blocks below the line Ability to increase U/P* was sharply limited before cortisone as compared with the normals The one apparent exception to this among the normals (A K) should be mentioned In this instance, as a result of a high previous salt intake, control concentration was so high that presumably maximal urinary

* Although serum sodium did rise 3 to 8 mEq per L during the experiments urinary sodium concentration changes were so much greater that U/P changes reflect essentially changes in urinary concentration

concentration of sodium had already been reached prior to the experiment In the three patients who received cortisone slight to marked increase in urinary concentrating ability resulted It should be emphasized that prior to cortisone treatment impairment of capacity to increase urinary sodium concentration was present in all patients including the one not on DCA and this defect was not demonstrated in two normals receiving DCA

The administration of sodium para aminohippurate during determination of Tm_{PAH} in two patients resulted in a very surprising finding for which there is no apparent explanation Here sodium excretions following sodium loads given in lower concentration at slower rates of administration resulted in greater excretory rates of sodium than had previously been observed with the same patients with hypertonic saline both before and after cortisone In two instances this was due to both increased urine flow and increased urinary sodium concentration following administration of sodium para aminohippurate In another it was due to an unexpected diuresis with little change in concentration

Administration of water loads to these same patients before and after cortisone demonstrated in keeping with Dr Thorn s findings a reversal of the Robinson Kepler Power test in three or four patients

sodium concentration is relatively inflexible and second ability to increase urine flow is limited

The precise renal abnormality in adrenal insufficiency has not been defined but there is good evidence that the characteristic salt loss is due to *decreased* tubular reabsorption of sodium(16) Yet in adrenalectomized animals receiving hypertonic saline at a rate rapid enough to produce serum sodium concentrations significantly above normal *increased* tubular reabsorption in the presence of normal glomerular filtration rates were demonstrated(17) In our patients with Addison s disease who excreted loads poorly the percentage of filtered sodium excreted was definitely lower than in the normal subjects in whom this measurement was made under similar experimental conditions However the individual inulin clearances in the patients varied from low to normal values In addition it was not considered safe to attempt to achieve as high serum sodium

levels as were produced in the animal experiments cited above A close comparison of the two sets of data is therefore not possible One thing does seem clear If the inulin clearance measures the glomerular filtration rate, the failure to excrete salt was not due to diminished filtration of sodium The highest sodium excretion occurred in a patient with the lowest inulin clearance and one of the poorest sodium excretions occurred in a patient with a normal filtration rate

The data reported here, and those of others cited, support the hypothesis that the defects of renal salt excretion in adrenal insufficiency are the result of impaired *responsiveness* of the renal tubules to the homeostatic requirements of the moment Some conditions demand increased tubular reabsorption of sodium and other conditions the reverse The renal abnormality in respect to water is chiefly one of inability to reduce the proportion of filtered water which is reabsorbed, despite the demands for water excretion by the organism The delayed removal of salt following hypertonic saline infusion, the inability to respond to water diuresis and the hyponatremia following salt restriction can all be explained by these two renal defects of salt and water excretion in adrenal insufficiency

Cortisone appears usually to correct the defect of increased water reabsorption and to improve but not entirely remove the inability to *change* reabsorptive rate of sodium

DISCUSSION

Shock Were all the reported measurements made during the period of salt and fluid retention or were some determinations made during the period following withdrawal of ACTH?

Burnett The measurements were made among the normal group between three and five days after starting ACTH They were therefore studied during the period when one could expect maximum fluid and salt retention, and they did uniformly show salt and water loss following withdrawal of ACTH

Thorn To clarify this point, Dr Burnett you maintained your Addisonian patients on DCA is that correct? There was also one patient maintained on salt?

Burnett That is right In two of the patients on DCA there was reason to believe that they were just about out of the DCA because

pellets had been implanted twelve and eight months previously The patient who had had pellets implanted eight months previously went into crisis on the night following the test so there was fairly good evidence that she was out or almost out of DCA Two others were adequately controlled on DCA at the time of testing, whether in these the presence of the DCA conditioned the responses has bothered us right along From evidence of other observers it seems probable that DCA did not have too much effect on the results reported here, first, because it has been observed in animals that were not on DCA is that correct, Dr Pitts?

Pitts They had been on small maintenance doses of DCA but were untreated for a period of three to five days prior to an experiment

Burnett Other experimental and clinical observations similar to these had been observed in patients with Addison's disease who had not previously received DCA (18,19,20,21) However, it is clear that in observations reported here previously, DCA may have conditioned the response

Thorn But the essential difference in the untreated Addisonian is a greater reduction in glomerular filtration when he isn't prepared with DCA beforehand?

Burnett Yes

Thorn Are filtration rates uniformly down?

Burnett Filtration rate may be somewhat improved by DCA but we were able to find no instances where improvement was as striking as was observed with cortisone

Fremont Smith I would like to ask a question about this water excretion The Addisonians have a delayed response to water excretion or failure to excrete water normally As far as I can remember, this is the only condition in which edema does not occur Why don t patients with Addison's disease develop edema?

Thorn They do as soon as you give sodium chloride or anything that improves their capacity to retain sodium chloride

Fremont Smith Why don't they develop edema spontaneously the way any other person does who has a failure to excrete water normally?

Thorn Possibly patients with other diseases develop edema in conjunction with salt retention There is a limit to water retention, isn't there without accompanying sodium chloride?

Fremont-Smith Then it must mean that they don't fail to excrete their water if they don't become edematous They must either get rid of the water or become edematous If this is not so, then they don't take the water

Thorn They don't take the water Their intake of water is very low and they do eliminate it over the twenty four-hour period

Fremont-Smith They have a failure of thirst, and if you give an average or a slightly elevated water load to an Addisonian patient, if he does not vomit it or does not have diarrhea, he develops edema is that right?

Thorn No If you give him this test load of water which Dr Burnett describes he eliminates it but his diuresis is delayed and it comes on roughly after the twelve-o clock period The test runs from eight in the morning to twelve noon in the normal individual and the normal individual excretes 50 percent or more of his water load in the first three to four hours

Fremont Smith If he cannot get rid of it or if he does not get rid of it before drinking again will he develop edema?

Burnett Actually we see again and again that the patient who has been treated for Addisonian crisis is edematous the morning following his treatment

Thorn That is one of the reasons why DCA has been so difficult to administer in proper dosage because of the inherent underlying tendency to retain water Thus, if one adds a very potent sodium retaining agent to a patient who has a tendency to retain water, one can predict the development of edema in a relatively short time

Fremont Smith Has anybody seen an untreated Addisonian with edema?

Thorn Never

Fremont Smith They are dehydrated

Thorn But their total body water content per gram of tissue is high Their extracellular fluid is dehydrated

Fremont Smith The blood volume is down

Thorn The blood volume is down The water content of the tissue is not down

Fremont Smith It is a striking example of contrast because they have a failure to excrete water and yet they are dehydrated The

curious thing is that in a sense they are similar to the diabetes insipidus because the diabetes insipidus if left alone would get a diminished extracellular fluid and diminished blood volume but he saves himself by drinking to keep up with his kidneys The Addisonian develops the same condition because he does not take water isn t that right?

Thorn Yes

Fremont Smith It is rather striking because I cannot think of any other clinical condition where there is failure to excrete water normally where the person does not become or have a real tendency to become edematous

Thorn These experiments that Dr Burnett carried out are really heroic and extremely difficult experiments to conduct in these patients regardless of the fact that they are maintained with DCA since the injection of even small quantities of sodium chloride intravenously will oftentimes precipitate a fever of 105° or 106° that evening Salt solutions may be extremely toxic to Addisonian patients maintained on DCA alone

I might add one point of interest for those investigating endogenous creatinine methods in Addisonian patients There are striking changes in creatinine and creatine metabolism following cortisone therapy

Shorr How long do your experiments last?

Burnett The actual infusion of hypertonic saline usually lasts an hour The actual experiment is usually about a three hour procedure

Shorr Some time ago we became interested in the response of the urinary uric acid creatinine ratio following administration of a test dose of ACTH(22) We found that without any ACTH normal subjects frequently exhibited a rise in the uric acid creatinine ratio which was as large or larger than the total rise achieved with ACTH In other words something happens to this ratio in the course of just fasting which apparently is not associated with any regular fall in eosinophils but which may give a pseudo response in the ACTH test Indeed in Addisonians fasting brings out the defect in response of the uric acid creatinine ratio almost as regularly as does ACTH

We then repeated the studies without ACTH on normal subjects modifying the initial level of uric acid excretion by giving on the

day previous to the test two meals high in purines An individual who responds to fasting alone with an increase in uric acid creatinine ratio of 80 to 240 percent may after a day on high purine diet show practically no increase in the ratio Similarly when ACTH is administered to a normal subject the response may be either an increase in this ratio of 90 percent or following two meals of sweetbreads it may be reduced to an abnormal 2 percent

Another thing which became apparent from these studies was that both with fasting and after ACTH an increase in urinary pH usually accompanied the rise in uric acid creatinine ratio

Burnett I cannot answer those questions in reference to uric acid and creatinine but I do not remember any difference between Addisonians and the normals In regard to the pH we thought we had something early and measured pH in the urine but there was not any constant change either in the normal or the Addisonian group including the urinary pH after administration of sodium aminohippurate load

Shorr The time intervals in our studies were the same as those in the Forsham Thorn ACTH test and it may very well be these are longer than yours We have a two hour control period before the injection and four hours afterwards That prolongs the experiment beyond your time relationships

Shannon What is the time relationship of the whole test as you do it?

Shorr We run the test as follows No food after supper the evening before the test two hours of urine collection from six to eight o clock in the morning ACTH injection at eight o clock urine collection from nine to twelve noon We use the same time relations in the mock test

Burnett I doubt whether there is even that great a difference As I think back some of our ACTH experiments ran over to twelve or one o clock because we had trouble getting under way There was in these patients therefore a long fast

Ralli Did you say that one of the major defects in the Addisonian was salt loss due to a decrease in the reabsorption of sodium?

Burnett Under some circumstances

Ralli At what level of sodium ingestion would the decreased ability to reabsorb sodium take place in the Addisonian?

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Thorn. Yes.

Fremont-Smith. It is rather striking because I cannot think of any other clinical condition where there is failure to excrete water normally where the person does not become, or have a real tendency to become edematous.

Thorn. These experiments that Dr. Burnett carried out are really heroic and extremely difficult experiments to conduct in these patients regardless of the fact that they are maintained with DCA since the injection of even small quantities of sodium chloride intravenously will oftentimes precipitate a fever of 105° or 106° that evening. Salt solutions may be extremely toxic to Addisonian patients maintained on DCA alone.

I might add one point of interest for those investigating endogenous creatinine methods in Addisonian patients. There are striking changes in creatinine and creatine metabolism following cortisone therapy.

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Burnett Under some circumstances

Ralli At what level of sodium ingestion would the decreased ability to reabsorb sodium take place in the Addisonian?

Burnett I have not any idea I simply picture the Addisonian in a narrower range that is he is unable to conserve salt below a certain level of intake and unable to excrete excess salt above another given higher level of intake I don t know where you put those lines Do you Dr Thorn?

Thorn No They depend upon an intact kidney since renal disease complicating adrenal disease would alter the threshold I would judge from clinical experience that a normal salt intake consisting of 8 to 10 gm would be well within the capacity of the Addisonian keeping in mind that the potassium intake is very important in determining the threshold for sodium tolerance

Dock If you keep an Addisonian on a high salt intake for a long period of time does he again come into balance?

Burnett Not according to Reifenstein s study As I remember the patient with Addison s disease stayed in positive balance(20)

Thorn He stays in positive balance but again this will depend on the extent of sodium intake

Shannon He cannot stay in positive balance indefinitely

Burnett As I remember the data as far as they carried the experiment for four to five days they stayed in positive balance

Shannon A normal individual if you increase salt will be in a positive balance

Thorn He comes into balance ultimately with increased total body NaCl

Shorr Like the hyperparathyroid patient receiving calcium he is constantly getting more and losing more

Bott Dr Burnett would you mind reviewing the conditions under which you obtained a reversal of sodium and potassium that is while giving sodium para aminohippurate you found that potassium para aminohippurate was excreted?

Burnett Referring back to Figure 15 the pattern of electrolyte and para aminohippurate excretion in the urine before and after Tm determinations during the control period and after ACTH are shown It is apparent that on administration of the load before ACTH administration a larger amount of para aminohippurate appeared in the urine but that most of it was in association with sodium with not too great an increase in potassium excretion

although in some instances there was increased potassium excretion in the urine

Under similar conditions after ACTH was administered with the increased para aminohippurate following loading, the quantity of sodium was decreased as compared with the control experiment whereas the quantity of potassium was increased This phenomenon was observed in all patients with ACTH and cortisone that we studied We have no explanation for this phenomenon but it has occurred to us that this reflects the so called potassium diuretic effect of sodium solutions In our experience, however, this effect is not constant Hypertonic saline, sodium bicarbonate, and sodium para aminohippurate in some instances may effect increased potassium excretion but not always Under the stimulus of adrenal steroids apparently, however, it has been brought out

Bott But this particular example was in a normal individual?

Burnett The patient shown in Figure 15 is a normal individual We did not observe this effect in two patients with Addison s disease in whom we studied it following administration of cortisone

Ralli What were the serum levels of potassium in the Addisonian at the time that you were doing these tests?

Burnett They were normal

Darrow That fits in with the findings in dietary potassium deficiency If you put rats on diets low in potassium and also low in sodium chloride, you get practically no changes in the muscles It requires a load of sodium chloride to produce potassium deficiency with desoxycorticosterone also

Burnett Dr Donald W Seldin* has shown in animals on a low sodium intake that it is impossible to demonstrate increased potassium excretion on administration of DCA I interpret that this effect in some way is related to the one you have mentioned

Darrow That is the general belief

Luetscher It would be important to study the sodium potassium exchange after para aminohippurate during the later phase of ACTH or cortisone administration when sodium is being released

Burnett I am sure it would

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* Personal communication

Luetscher That might well show a reversion to normal A similar exchange of potassium for sodium was observed by Dr Elliott Newman(23) in patients with nephrosis when he measured the clearance of thiosulfate Sodium thiosulfate was given by infusion Patients with edema and with little sodium in the urine excreted potassium with the thiosulfate When sodium appeared in the urine normally thiosulfate was normally excreted with sodium The impairment of sodium excretion demonstrated under this stress is usually visible in ordinary daily balances

Janeway We have seen regularly in nephrosis that we get a big potassium diuresis during infusions of sodium salts

Burnett You have observed it with thiosulfate and we have with sodium bicarbonate with patients with nephrosis It seems to be a very common phenomenon regardless of the sodium salt which is employed

Gaunt I wonder if anybody has considered the possibility that potassium diuresis when it occurs after a heavy sodium load might be due to action of the posterior pituitary hormones If you give enough sodium to obtain release of antidiuretic hormone you might expect to get possibly the excretion of potassium

Burnett That may well have been a factor in some of our experiments with ACTH because we know that certain lots were contaminated with posterior pituitary It also appeared however with cortisone and in these instances one would have to assume that cortisone stimulated the release of antidiuretic hormone

Gaunt Another point I would like to make relates to the question of what would happen in case the salt load followed the water load I saw some figures recently that have been collected by Dr Drill and Dr Hays at Wayne University* They found as everyone else has that if you give adrenalectomized animals a water load they cannot excrete it normally If you give them a load of normal saline they cannot excrete it normally But they worked with various concentrations of salt in their water load and found a point roughly as I remember it about 0.4 percent sodium chloride at which the adrenalectomized animal would excrete just like the normal animal This impressed me because we all have had so much difficulty getting that response back to normal They did it just by adjusting the concentration of salt in the water

* Unpublished data

Ralli We have been determining the critical requirement for sodium chloride in adrenalectomized rats on a high pantothenic acid intake. As you may recall we have observed that if the diet of the rat following adrenalectomy is supplemented with large amounts of calcium pantothenate(24,25) and a 1 percent solution of NaCl as drinking water survival is greatly prolonged. If the rat is given *no* NaCl the pantothenic acid content of the diet has no effect on survival and if the diet is deficient or normal in its pantothenate content 1 percent NaCl will not prolong survival and the rats die in an average of eleven days after adrenalectomy. In the present experiments sixty day old rats were adrenalectomized and were placed on measured amounts of NaCl and a diet containing 4 mg of calcium pantothenate per 10 gm of diet. The first group were given 1 mM of NaCl daily (6 ml of a 0.167 Molar solution). A second group received 3 m mols of NaCl daily (18 ml of a 0.167 Molar solution) and a third group received 5 mM of NaCl daily (30 ml of 0.167 Molar NaCl). Distilled water was provided in a separate drinking bottle to assure a sufficient fluid intake. In the adrenalectomized animals receiving the NaCl solutions of 1 and 3 mM survival was sharply limited the median survival time being nine and fourteen days respectively. When the NaCl concentration was increased to 5 mM survival was increased but it still did not equal the survival in adrenalectomized rats permitted a 1 percent solution of NaCl *ad lib*.

The experiments are still in progress and when we have determined the daily amount of NaCl necessary for prolonged survival in adrenalectomized rats on the high pantothenate intake we plan to study whether the concentration of the NaCl solution conditions the animals requirement under the circumstances of these experiments.

Gaunt In the maintenance experiment the animals would get along still better with more than 0.5 percent sodium chloride would they not?

Ralli They would.

Gaunt What I was speaking about is an acute experiment — how to make an adrenalectomized animal excrete simultaneously a salt and water load. If you get the salt and water in proper proportions according to Drill and Hays the animals excrete it at a normal rate whereas in other proportions they do not.

Thorn It might be worth while for the group to keep in mind correlated experiments that might be helpful. The control of the

sodium chloride concentration in the sweat has been shown to be modified significantly by alterations in adrenal hormone level(26) Frawley(27) in our group recently has shown that within a certain range of serum sodium level the sodium chloride concentration in saliva provides another means of demonstrating the effect of adrenal steroids In analysing these problems, I wonder whether any evidence has ever been obtained which might suggest that the kidney also "secretes" sodium

Shannon I think that is pretty close to the point I was going to make in relation to Dr Burnett's data Our information on the sequence of processes which condition the formation of urine in the mammalian nephron is wholly inadequate for one to answer the many questions raised by experiments such as have been presented

Burnett Do you think we can ever do that without getting back into the tubule?

Shannon I believe one can obtain a part of the solution there, that is by an extension of the studies by Oliver, Richards and Walker, but under more physiological conditions It is quite essential to know whether the absorption of inorganic ions in the proximal tubule proceeds at a more rapid rate than the movement of water This must be settled before one can attempt a placement of certain functions in the proximal or distal tubule I don't see any other way of doing it than by tubular punctures in wholly physiological preparations

Pitts We tried one possible means of attack on the problem using hippurate infusions with the view, if we could markedly lower filtration rate without affecting tubular transport, that we might be able to demonstrate secretion of sodium We couldn't demonstrate secretion

Burnett We have been struck with one fact in all of these experiments Originally some patients were studied simply on a general ward diet We soon found, however, that we had to have them on at least a week of constant intake before we could get any kind of reproducible result All the patients shown here had been on a constant intake for a week prior to the control test and during the time of hormone administration If this program is not adhered to the results mean absolutely nothing

Pitts What is the effect of cortisone or ACTH on phosphate excretion without loading?

Burnett It increases but not as markedly as it did after loading There also was an increase in phosphate excretion during the day to day measurements

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THE EXCRETION OF SODIUM IN RELATION TO GLOMERULAR FILTRATION

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I WOULD like to say a few words about the relation of glomerular filtration to sodium and water output If glomerular filtrate is suddenly reduced by 8 or 9 percent, as when one changes from lying down to standing up, the salt and water outputs drop to a third or fourth of what they had been This is what one would expect Nothing has happened to the tubules to inform them of any change so they continue to reabsorb, as nearly as they can, what would approach the same amounts of salt and water as were being reabsorbed before, so there is a great deal less remaining to be excreted That is an acute condition If this kept on for very long it would result in an accumulation of surpluses of salt and water, and the tubules would somehow be informed of this and would start rejecting more, so that the output would come back to where it was before In other words, a short-lasting, small reduction in glomerular filtration would be expected to produce a much greater acute percentage reduction in sodium and water outputs, which it does, whereas a long-lasting, even great reduction, in glomerular filtration would not be expected to produce any chronic reduction in salt and water output if the tubules behaved normally The question is how to test this by producing long-lasting reductions in glomerular output while still having normal kidneys and body fluids If one reduces filtration by hypophysectomizing an animal, brings it down to half the normal, there is no retention of either salt or water The animal stays in balance because his tubules reject a larger fraction of the filtered salt and water, and he can get rid of suddenly imposed loads of salt just as fast as the normal animal can, but that still isn't quite the answer

One of the things we have been doing during the past year is to prepare animals so that we can produce a permanent fall in filtration rate with the kidneys undamaged and without any accumulation of salt or water This was done by explanting the bladder trigone so we could collect from the two kidneys separately These

animals survive for many weeks or months If then a ligature is put on one renal artery so as to restrict very slightly the blood flow through that kidney there will be a great reduction in the salt and water output of that kidney, with a corresponding increase in the salt and water output of the other kidney The output, with intake constant, is the same as it was before, the animal stays in balance indefinitely The kidney that was constricted does not know that anything has happened to it In fact, nothing has happened to the composition of the blood, because the other kidney prevents any accumulation from occurring, so the constricted kidney continues to reabsorb at somewhere near the same rate that it would have if there had not been this slight reduction in filtration The fact that the companion kidney increases its sodium output might be regarded as evidence that some accumulation has occurred This cannot be definitely answered at present, the important point is that any presumptive accumulation is negligible compared to what would have occurred if the output of both kidneys had suffered the same percentage decrease as that of the ligated kidney

I will cite a few specific cases If one puts on a ligature of just the proper tightness, and a few days later determines inulin and PAH clearances and sodium and water outputs of the separate kidneys there will be no measurable fall in the clearances of the ligated kidney, but a fall of 50 percent in sodium and water outputs At the same time the sodium and water outputs of the other kidney have gone up so that the two are putting out the same as they did before These animals are kept on constant intake In other words a *unilateral* fall in filtration rate too small to be detected by our methods will result in a unilateral 50 percent fall in sodium output *and that will last indefinitely*, whereas if *both* kidneys had been so ligated as to reduce their filtration rates by 5 percent, there would have been no change in the salt or water output of either kidney after a short time

If we make the constriction somewhat greater by progressive tightening so that the PAH and inulin clearances are obviously reduced, say to 80 percent or 70 percent of normal, the sodium and water outputs of the constricted kidney drop to about 3 percent of the companion's outputs The companion kidney's outputs have increased so that the total outputs remain the same With a still further constriction, but not complete obliteration of the artery, since one can still feel the pulse distal to the constriction, urine output ceases entirely from this kidney, although it still has a blood

flow I don't know what fraction it is because we have no way of measuring it The other kidney then puts out the same amount originally put by the two kidneys If we take an animal which has a constricted kidney with a filtration rate of 70 percent of normal but with only 3 percent of the sodium coming out of that kidney and now remove the good kidney, the remaining ligated kidney will of course increase its PAH and inulin clearances but is prevented by the ligature from increasing them as much as happens after a unilateral nephrectomy as ordinarily done The inulin clearance of the remaining ligated kidney thus rises somewhat but never reaches the level of two normal kidneys However the salt output rises so that within three days after the removal of the good kidney the salt output of the remaining kidney is nearly normal, and eventually becomes normal, i e , it is taking care of all the salt that both kidneys had been doing This emphasizes what has perhaps not always been adequately emphasized, the distinction between transitory and long lasting changes in filtration rate as they affect salt and water outputs To me it seems only to be expected that a sudden small drop in filtration rate would produce a great drop in salt and water outputs but that drop would not last very long In a short time it would be right back to normal, but when the other good kidney prevents a surplus from accumulating then this small drop of say 5 percent in filtration rate will permanently reduce the salt output of that kidney But when you take the other kidney out then the salt begins to pile up even though it isn t easily detectable by analysis, the tubules promptly responding by rejecting a higher fraction of the filtered salt than they did before

DISCUSSION

Pitts When one renal artery is moderately constricted, does the remaining kidney increase its filtration rate appreciably?

White Yes, if the constricted kidney has been reduced in its filtration enough to be sure about it Of course we cannot be really sure of drops in filtration today as compared with two weeks ago unless there is at least a 15 percent drop However, the sum of the two clearances will be somewhat less than before, the companion kidney does not show as much of an increase as there is a decrease in the constricted kidney

Pitts Would you interpret that as meaning that perhaps the somewhat discredited concept of glomerulo tubular balance has an element of truth in it?

White The actual immediate response of the tubules to a change in filtration rate without change in composition of blood or filtrate is somewhere between that of continuing to reabsorb, within limits the same amount of salt per unit time as before, and of reabsorbing the same percentage of the filtered amounts as they did before

Selkurt Another interesting speculation on your experiment is the fact that when you begin to ligate the renal artery you reduce the pulse pressure without reducing mean pressure In 1913 Gesell (*1*) came out with a paper that indicated that the pulse pressure might be an effective means of varying the salt and urine output His experiments demonstrated that when the pulse pressure was reduced there was no change in renal blood flow There was a drop in renal output of chloride It may be possible that in your experiment the first effect might be the reduction of pulse pressure with its concomitant effect on the urine volume and sodium excretion That could go on with very little change in filtration rate and blood flow particularly since the circulatory autonomy of the kidney tends to maintain the flow rather well even as you begin to impair the arterial input It might be a factor to consider in that experiment

White I doubt if we had any very significant fall in pulse pressure distal to these lightest constrictions, but whether we did or not, the fact remains that there was no measurable fall in filtration rate We felt that both ureters should be accessible because we wanted to be sure that we had the same errors of collection in the two These are not twenty four-hour collections, but the animals were on a constant diet We chose arbitrarily a three hour period from ten o'clock in the morning to one o'clock in the afternoon, always the same time of day, always in the postabsorptive state no food since five o'clock the evening before The outputs then are fairly reproducible from one day to the next, although there is considerable fluctuation But you see we always have the other kidney for control It is actually easier to transplant the two ureters than one, because when the trigone is transplanted the ureteral orifices are still in the bladder mucosa they do not slough out or become stenosed, and no trouble is encountered with ascending infections I don t remember now whose operation that is but it is not original with us It makes a very satisfactory preparation The animals survive a long time without any ascending infection

Shannon How do you make the urine collections in those preparations?

White Just put a catheter in them

Shannon The animals dont develop infections?

White We dont put it in very far We have done it in two ways Sometimes the animal stands in a canvas sling Then somebody has to hold a little cup up against each ureteral orifice with or without suction but it usually works perfectly well if you just put in a small catheter I dont mean we go up to the kidney pelvis just an inch or so

Pitts How do you keep from getting superficial skin erosion skin infection and breakdown? That was our problem in the use of such dogs a number of years ago

White There has been no difficulty with the wound That is quite different from our experience of a number of years ago when we explanted the ureters into the separate flanks but when the bladder mucosa is there it heals up well I dont mean that all of them get along with a perfectly uneventful postoperative course but most of them do I have not done these operations myself they were done by a competent surgeon Dr Barber Mueller It is apparently a relatively simple procedure in such hands

Darrow What worries me is why the tubules on two different sides should be adjusted to different rates of absorption Do you calculate terms of load from filtrate? Is there anything common? After all they are presented presumably with the same sort of fil trate arent they?

White Yes presumably they are getting the same kind of filtrate

Darrow How is your kidney wise enough to do different things on the two sides of the body?

White Both sides are being perfused with the same perfusate and the tubules on both sides are being exposed to essentially the same fluids as before we put on the constriction So how do they know that anything has happened? Why should they change their behavior?

Darrow Eventually they do

White No they do not What I mean is they continue to do as nearly as they can the same thing as before

Darrow At first but not later

White They keep on permanently so long as the companion kidney remains If you permitted an accumulation of salt to take place then they would not keep on They would finally realize there is too much salt here and quit taking back so much of it Don t ask me how they recognize that That is a question of a different category

Lauson Do you believe this is relatively independent of hormonal factors?

White We know that in this case both kidneys are in the same dog They are subjected to the same hormonal influences unless we want to postulate that one kidney is itself making a hormone that the other one is not making That is the only possible difference

Lauson Would this be an intrinsic renal adjustment of the good side to the necessity for excreting extra salt on that side because of the intrinsic inability of the opposite side to excrete its share?

White That is right because if it did not put out more salt then salt would accumulate It probably does accumulate at first It seems to me this is quite analogous with our problems in the regulation of respiration Why do you start breathing harder when you make more CO_2? Because if you did not the CO_2 would pile up until you got into trouble One step nearer an explanation is of course that the respiratory center has responded to the increased CO_2 tension While we all hope to know some day more about how the insides of cells work we do not need to know the intimate details of how a response is effected in order to know that it occurs As soon as salt or CO_2 has accumulated by some threshold increment the behavior of the tubules or of the respiratory center respectively changes

Ralli Is this not largely due to the difference between the filtration rate in the two kidneys?

White The tubule is being advised that the composition of the plasma and filtrate is relatively unchanged so it continues with a relatively unchanged reabsorptive behavior Unchanged tubular reabsorptive behavior toward salt is here defined as that which affects the reabsorption of a constant amount of salt per unit time subject only to the limitations of a decreased amount available for reabsorption and of the practical impossibility of extracting the last residuum of salt the tubule is set to reabsorb the same amount of salt per unit time as before if it is given the opportunity Such

tancy of behavior depends on a constancy of environment but d not be altered by changes in filtration rate only It does not n reabsorption of a constant percentage of the filtered salt a constant amount per unit volume of filtrate nor a constant of total osmotic work As mentioned before complete con cy so defined is not actually realized somewhat more salt pes on decreased filtration than that predicted on this basis somewhat less on increased filtration

all Would it not be reasonable to say that this change in ation rate in the kidney might set about a series of reactions ch would have a wider effect than would be anticipated

Vhite That is possible of course I dont visualize what those be I can "explain it to my own satisfaction by saying that if didnt happen salt would pile up I do not mean to endow the ules with the faculty of anticipating changes in salt level I only n that as soon as some slight increase in level has occurred the ules in some way recognize this and depart from their constancy alt reabsorptive behavior as defined here they now begin to osorb less salt per unit time even if filtration rate has not nged since the plasma and filtrate are now saltier The result hat more salt is excreted and accumulation of any great surplus revented The unilateral ligation experiments enable us to see at happens when only one factor filtration rate is changed e the presence of the companion kidney prevents the surplus ch would otherwise accumulate In this case the state of con it reabsorptive behavior as defined above is approached but no longer holds when conditions are such that the usually duced surplus can develop as when fall in filtration rate is teral or after the companion kidney in these experiments is noved

Darrow Either you have or you do not have evidence that as tubular reabsorption changes on the good side an analogous nge takes place in the other kidney s tubules What I have in nd is that one could calculate the tubular load of sodium from glomerular filtration Then if one assumes that 80 percent of filtrate is reabsorbed in the proximal tubules one has a value the reabsorption in the distal tubules on the two sides If the tal reabsorption is at the same rate on the two sides then the nary excretion should be explained by the same rates of distal bsorption but different distal loads on the two sides Otherwise

one might have to assume a local adjustment in the rate of distal reabsorption dependent on the different loads and other local factors If one can explain the rates of excretion in terms of glomerular filtrates one has a generalized adjustment on the two sides that are referable to such general factors as hormones

White I think that is right

Luetscher Does any accumulation of water and sodium actually occur?

White We do not yet have for comparison any observations made immediately after the ligation

Luetscher Two important questions arise First does the filtration rate go up on the unimpaired side equally with the diminution on the side on which the ligature was placed? Second does the body weight or extracellular fluid volume go up at any time so as to provide a stimulus to such a readjustment as Dr Darrow suggests?

White The body weight does not go up

Bradley Do the two kidneys respond differently to a sodium load?

White Yes The good kidney still puts out the major fraction of the salt

Pitts But both will put out more salt?

White Yes If in the unloaded state the ligated kidney is putting out 5 percent of the sodium it will still put out 5 percent when loaded i e the two kidneys show the same percentage increase in output on loading

Pitts Isn t it merely a quantitative conceptual difficulty? If both kidneys to begin with are reabsorbing 99 percent of the salt and water and you cut filtration rate slightly on one side so that that kidney now reabsorbs 99 8 percent of the salt and water the other kidney has only to decrease its absorption to 98 2 percent for the system to come into balance again

Gilman I think Dr White s experiments serve to remind us of the fact that the kidney is an end organ and an end organ charged with tremendous responsibilities It has a large amount of work to do All of the reabsorptive mechanisms that we have discussed are active transport mechanisms and require the expenditure of energy Also we should remind ourselves that the subject of this Conference has to do with the hormonal control of renal function

Although we can imagine some autonomous function on the part of the kidney, certainly its homeostatic mechanisms must be under the control of a gamut of endocrine and possibly other types of controls It is not surprising therefore that when two kidneys are receiving the same humoral messages their tubular function would tend to be equal insofar as the extent to which transport mechanisms are carried out Under these circumstances when you decrease the load to one and it is still under the same humoral influences as the other, it is going to attempt to do the same reabsorptive job that the unoperated kidney does It is not going to do a complete job of reabsorption of electrolytes because all sorts of things are going on in the tubule which will prevent complete reabsorption of electrolytes despite the diminished amount of filtrate The most obvious of these is the presence in the tubule of substances which are not actively reabsorbed and their presence in the tubule prevents reabsorption of sodium, chloride and other electrolytes Probably urea is the outstanding substance in that category Therefore we can expect these tubules receiving a reduced load to behave in the same way and to have essentially the same reabsorptive capacity as the normal kidney but we cannot expect complete reabsorption because of the osmotic effects of substances which are not actively transported

Shorr I wonder whether we don't have to consider the possibility of an influence by locally produced humoral factors because this is essentially a hypertensive kidney

White There is no hypertension in these dogs

Shorr Is there not a temporary hypertension?

White I can not answer as to a transitory hypertension The measurements were made about once a week and there was no hypertension seen

Lauson I would like to raise two or three specific questions If one had a 30 percent reduction in glomerular filtration rate on the affected side and at that time had this modest increase in salt excretion on the good side, one would assume that the kidneys were not under excessive stimulation by endogenous desoxycorticosterone like hormones Now what would you anticipate would happen to the affected kidney with excessive DCA administration? Could the affected kidney be made to reabsorb more NaCl under these conditions? It seems to me this has a bearing on the question

of whether excessive DCA like activity does exist in certain states in which the glomerular filtration rate is low such as congestive failure and the nephrotic syndrome and if it does exist could such adrenal hyperactivity cause more complete NaCl reabsorption than is already taking place due to lowered filtration rate? I wonder if NaCl balance could be restored without edema formation if one had a chronic constriction like this on both sides giving a chronic reduction in glomerular filtration of about 30 percent

White We didn t carry out bilateral constriction but it is well known that Goldblatt dogs do not develop edema We did not want to produce hypertension we thought if we ligated both kidneys we would probably get hypertension which would confuse the issue I don t know whether 30 percent reduction in blood flow will usually produce hypertension or not even if it is acutely put on Does anybody here know? There are only two papers that I have found one by Corcoran and Page(2) and one by Blalock(3) years ago It is hard to cut down renal blood flow it gradually rises again and after a while is almost back to normal but they still have hypertension The question as to how much you have to reduce the blood flow to the dog s kidney to get hypertension is one to which I do not know the answer

Pitts It seems to me that Dr White in his chronic experiments has very beautifully shown that a small reduction in renal arterial caliber and filtration rate on one side can disturb balance The work of Blake Wegria *et al* (4) at the College of Physicians and Surgeons has shown that an increase in venous pressure accompanied perhaps by a small change in filtration rate can likewise produce this same imbalance O W Sartorius* has shown that an increase in ureteral pressure can likewise throw the system out of balance with only a very small change in filtration rate It is true that all these effects might be mediated through some change in the pulse pressure in the kidney but the common factor to me would appear to be the change in filtration rate The question is how can one reduce pulse pressure without producing some minor but highly significant change in filtration rate

Dock In Blake s experiments the filtration rates were unchanged

Pitts That is debatable if you examine their data closely

Selkurt I would like to think that in similar experiments that

* Personal communication

we did(5) the obstruction to blood flow was small However we frequently obtained decreases in filtration rate, though slight in some cases I was very interested in the remarks that Dr White made They seemed to indicate that very small changes in filtration

common ground on these phenomena where the whole explanation

and better tubular reabsorption of the reduced load

White As I remember Katz' group(6) showed that chronic elevation of renal vein pressure does not result in any permanent changes in sodium output

Pitts He did not do the two separately?

White No

Pitts I think repetition of his experiments using your technique would give an entirely different picture

White Yes I would expect to find essentially the same changes with unilateral renal vein constriction as with arterial I think that whereas slight bilateral changes in filtration rate will temporarily produce big changes in output, the changes in output won't last long In a short time the total output goes back to normal even though the rise of venous pressure persists whether it be on one

monal influences unless it is renin doing it and renin apparently would work the other way because presumably there should be more renin in the ligated kidney than in the other – then the effects should be permanent as they turned out to be

Gaunt Dr Pitts I would like to mention some observations of Dr Boss and Dr Osborn in our laboratory(7) These show an effect of cortical hormones on water reabsorption under conditions of a chronically low filtration rate The work was done on hypophysectomized rats

Several years ago Chen and Geiling(8) showed that the hypophysectomized rat despite its potential diabetes insipidus could not excrete a water load normally In extending the work we found that the urinary loss of fluid after water was given by stomach tube was no greater than the insensible loss(9) We tried to repair the deficiency with various hormones given singly and in combination The only thing that helped was adrenal cortical preparations but with them it was difficult if not impossible ever to restore a completely normal diuretic response to water The observations of Boss and Osborn show why A few days after hypophysectomy the glomerular filtration rate fell to about half of normal This was accompanied by a great increase in the tubular reabsorption of water The conditions of the experiment were such that under a water load 10 percent of the glomerular filtrate was recovered as urine in normal animals Only about 4 percent of the filtrate was recovered in the hypophysectomized animals and as already indicated the filtrate itself was of low volume When cortical extract was given the hypophysectomized animals in acute experiments 10 percent of the glomerular filtrate could be recovered as urine as in normal animals but the urine volume itself was only about half normal The reason apparently was that the cortical hormones did nothing to restore the filtration rate They restored reabsorptive rates to normal but the urine volume was limited even after treatment by the chronically low filtration rate and in an amount proportional to the reduction in filtration The failure of cortical hormones to affect filtration accords with the work Dr White reported earlier on the hypophysectomized dog As seen here however these hormones can affect reabsorptive processes in the absence of the pituitary

Selkurt Does sodium excretion parallel the water?

Gaunt The flame photometer broke down in the middle of the series and I cannot say with certainty We got only some of them and they indicated that there may be a disproportionately high sodium excretion associated with the increased urine volume induced by cortical extract We will know definitely about that soon

Lauson I would like to describe some observations on a series of around the clock measurements of endogenous creatinine clearance and of excretions of sodium and water which strongly suggest that glomerular filtration rate plays an important role In a five year old girl with nephrotic syndrome the creatinine clearance was raised

acutely on successive days by the infusion of albumin, which is a relatively simple way of changing the circulation without doing much to the endocrine system In this study there was a striking rise in sodium excretion and diuresis associated with relatively modest increases in glomerular filtration rate as estimated from the creatinine clearance We have been inclined to feel that this effect is more an intrinsic renal affair than an endocrine one, but of course one cannot be entirely certain of this

Luetscher The injection of concentrated human albumin in nephrosis leads to a series of changes which are very complex In addition to the increased creatinine clearance, there are increases in plasma albumin concentration, in plasma volume, in urine flow and in serum sodium concentration No one of these factors alone appears to guarantee the normal excretion of sodium in the urine To complicate the matter further, Dr Deming and I have noted a fall in the sodium retaining activity of the urinary corticoid fraction during and after albumin-induced diuresis in a few patients with nephrosis

Lauson These are the acute manifestations within the same day I would be the last to deny that long term adjustments are made These probably include such factors as ferritin which Dr Shorr has already discussed

Luetscher Another problem in the study of nephrosis is that various patients respond quite differently to the same stimulus In Figure 18, you can see some variations in the effects of albumin on the inulin clearance In these acute experiments, we were impressed by the large, rapid changes in glomerular filtration which were followed only after some time by the sluggish and reluctant appearance of sodium in the urine We had the feeling that an increased glomerular filtration rate was an important factor, but not the only one concerned

There is again a variation in response, which depends on the situation If the serum sodium concentration is very low, there is usually a clear-cut initial diuresis of water, during which very little sodium is excreted The serum sodium concentration increases Such a loss of water without sodium has been observed at the very beginning of spontaneous diuresis and after the administration of albumin A striking example is seen in Figure 4* during treatment

* See p 24

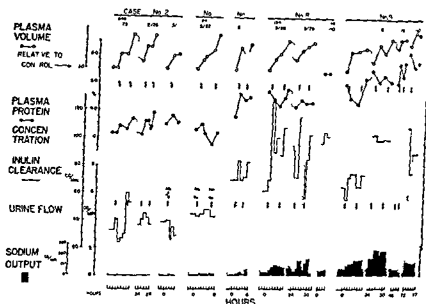


FIGURE 18

Effect of injections of albumin on several factors affecting the excretion of sodium

Each small arrow indicates the injection of 25 gm of albumin Note that the scale of urine flow and sodium output are magnified ten times in comparison with the inulin clearance Reprinted from *J Clin Investigation 29 896 (1950)*

of nephrosis with cortisone when water was lost without sodium and again during administration of ACTH when the patient lost *several kilograms of fluid before any sodium appeared in the urine* All of these patients had a considerable reduction in serum sodium concentration In this setting a release of water regularly appears as the first event in a diuresis During albumin or cortisone treatment it is not invariably followed by complete diuresis however and the serum sodium concentration may rise to normal or higher without the appearance of sodium in the urine

On the other hand if the serum sodium level is nearly normal before the diuresis the initial water diuresis is not seen and complete elimination of edema may occur with little change in serum sodium concentration

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ENDOCRINE FACTORS IN THE UTILIZATION OF GLUTATHIONE

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I WOULD like to report certain of our experiments* which may bear on our ideas of the mechanism of action of the renal tubule Certain of our experiments have consisted of moderate saline loading of the dog with physiological sodium chloride so that there was a constant urine flow of about 2 5 ml and a relatively constant excretion of sodium Then a mixture of glutamine and sodium glutathione (1 8 and 5 0 gm respectively per 500 ml of saline) was infused with the saline Within fifteen minutes there was a *sharp decline in the rate of urine flow* and within sixty minutes the urine flow was reduced to about one tenth of its original amount The creatinine clearance either increased or remained constant The sodium reabsorption was about the same as the water reabsorption We have not had a dissociation of the two

Except for one possible catch our series of experiments do show a very definite relationship between the metabolism of glutathione and the reabsorption of sodium by the kidney The catch is that glutathione contains a small amount of pyrogen when solutions of glutathione are injected into a rabbit there is a small temperature rise I should mention as a part of the further evidence for the relationship of glutathione and sodium transfer that Grunert and Phillips(1) in experiments with alloxan diabetic rats on low sodium diets have found that those rats develop low sodium levels and glutathione levels such that there is a correlation between the two levels

In Figure 19 is an illustration of what happens as measured by our methods of analysis(2) following the treatment of patients with ACTH This patient received only 40 mg of ACTH daily There was a gradual decrease of the levels of glutathione over a period of several days and at the same time there was an increase of

* These studies were supported by grants from the United States Public Health Service F H Tyler H P Plenk J R Kimmel, C K Olson S F J F C Wu and G M Christensen were collaborators in the work reported here

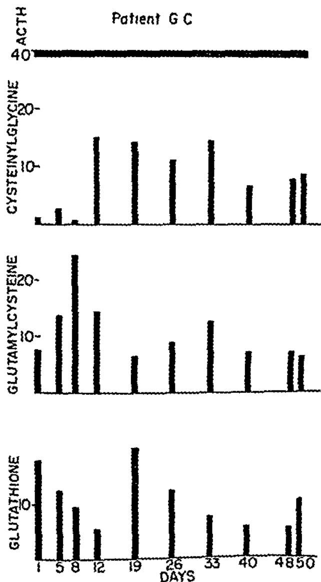


FIGURE 19

[illegible] ACTH to a patient (leukemia) on levels of gluta[illegible] The levels are expressed as mg [illegible] to a hematocrit of 50 percent

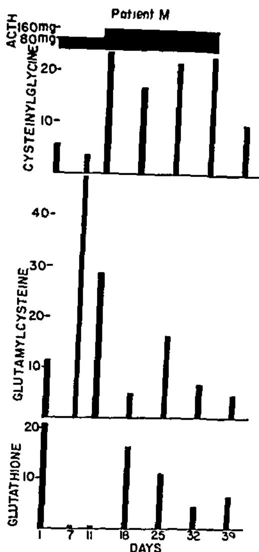


FIGURE 20

γ glutamylcysteine As we have reported(1), this fraction is characteristic of diabetic bloods The increase of this fraction also corresponded to the period when excess creatine was found in the urine

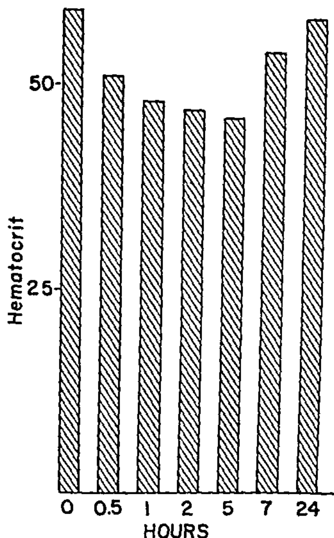


FIGURE 21

Effect of glutamine upon the hematocrit of a dog 1 8 gm glutamine in 500 ml saline was given intravenously to a 40 kg dog In twelve experiments the average hemodilution was 32 ± 12 percent at 4 hr following administration of the glutamine

Since γ glutamylcysteine is glutathione less glycine glycine may have been used in the synthesis of the extra creatine Studies of another patient are given in Figure 20 The dosage of ACTH was larger and glutathione disappeared from the blood

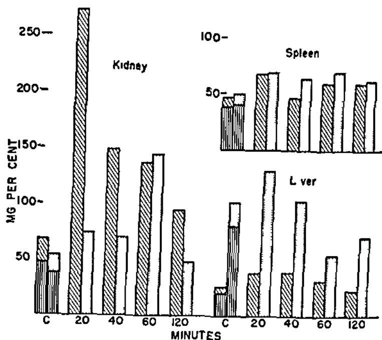


FIGURE 22

Effect of administration of glutathione on the levels of glutathione and the products of its hydrolysis in tissues of the rat Mean values and standard deviations for cysteine and glutathione of a control group respectively are indicated in the C T enously at the f gluta 0 01)

When glutamine was infused in the dog there was a sharp drop of the hematocrit most of the decrease occurred in the first fifteen minutes In the experiment shown in Figure 21 infusion was stopped in about an hour and the drop of hematocrit continued for several hours and returned to normal within twenty four hours

Many of our studies have been handicapped by lack of knowledge of the fate of glutathione and the factors influencing the utilization of glutathione Therefore a rather extensive group of studies were undertaken In the first experiments glutathione was given intra

found that the glutathione of muscle disappeared following the administration of ACTH and it appears that the glutathione had

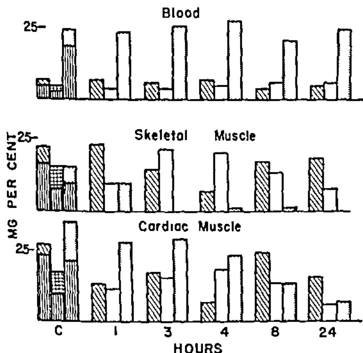


FIGURE 23

neally
ervals
f the
(C)
column Cysteinylglycine and γ glutamylcysteine are found in those tissues (blood and muscle) but are not found in liver kidney tissue or the spleen The decrease of glutath one and the increase of γ glutamylcysteine in muscle tissue were found to be highly significant No significant changes were found in spleen kidney or liver t ssue

been converted to glutamylcysteine (Figure 23) There was some increase of the γ glutamylcysteine in the blood with the short term administration of ACTH In cardiac muscle there was very much the same type of reaction (Figure 23)

Shorr How much ACTH was given?

Binkley We gave 2 mg to each rat

Thorn You believe that ACTH increases the utilization of gluta thione by the kidney?

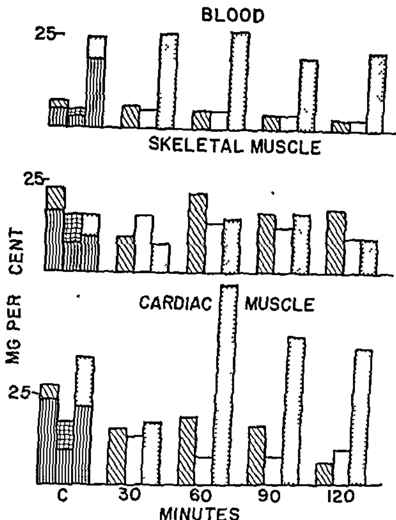


FIGURE 26

Effect of administration of insulin on the levels of glutathione and the products *of its hydrolysis in the tissues of rats* Two units insulin were given subcutaneously to rats and the groups (five or more) were sacrificed at the intervals indicated Levels of cysteinylglycine γ glutamylcysteine and glutathione (mean and standard error of controls mean of experimental groups) are indicated The changes of glutathione in cardiac muscle were highly significant The increase of glutathione in blood was found to be highly significant when similar experiments were carried out with rabbits (*Serial Samples*)

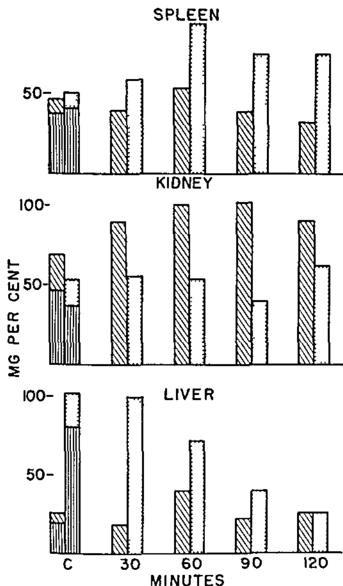


FIGURE 27

Effect of administration of insulin a continuation of the study of Figure 26 Levels of cysteine and glutathione are indicated The changes of glutathione in spleen, cysteine in kidney and glutathione in liver were significant (P=<0.01) When amounts of insulin as great as 10 units were given there was a decrease of cysteine in kidney at 120 minutes

and tissues other than muscle start building up higher and higher levels of glutathione The administration of insulin appears to mobilize this extra glutathione from the liver (Figure 27) All the glutathione in the liver may be removed within one hour after administration of larger amounts of insulin At the same time, the kidney was obviously utilizing larger amounts of glutathione since the concentrations of cysteine were increasing It would appear that insulin does mobilize glutathione first, by increasing the utilization by muscle tissue, and second, by stimulating the removal from the liver so that all tissues are allowed to take up excess glutathione very much as when glutathione was administered

Thorn You have not tried to balance ACTH and insulin on liver glutathione? Do you suppose you would observe antagonistic action of these hormones on this system?

Binkley We have not tried balancing the effects

Janeway Do you think glutathione is synthesized? Is it synthesized and built up in the liver and then taken from the liver and utilized in other places? Or is it built up in the kidneys or any site, such as the muscles and other tissues?

Binkley Our concept is that it can be synthesized in all tissues except the reticuloendothelial system The adaptation in crisis depends upon how much there is in the liver and the synthetic ability of the liver There is definitely transportation in the red blood cell

Thorn I wonder whether the hematocrit change in part might not reflect change in red blood cell size

Binkley They tell me not

Thorn There is a real entry of fluid into the vascular bed

Darrow Is there any change in the electrolyte concentration of the serum?

Binkley A slightly reduced concentration of sodium

Shannon What would be the average plasma dilution you obtained with these various doses?

Binkley Something around 15 to 20 percent

Dock Did you give glutathione and glutamine together?

Binkley In the experiment in Figure 21, glutamine was given Somewhat better effects were observed with glutamine plus glutathione

Shannon What are the normal concentrations of these substances in plasma?

Binkley Of glutamine, it carries approximately 10 mg percent, of glutathione 25 to 40 mg percent

Shannon What changes result from the dosages you administer?

Binkley We cannot change the glutathione level by the administration of glutathione, the tissues take it up very promptly I should not say we cannot affect blood levels In conditions like operative shock on rats the levels of blood glutathione are found to fall to quite low levels We can bring those levels back to but not beyond, normal levels by the introduction of glutathione

Thorn Have you ever tried raising the level in a nephrectomized animal by administration intravenously under these circumstances?

Binkley. No

Thorn I just wondered whether you thought the liver is the organ which defines the level

Binkley The liver I think to a large extent is a reservoir of glutathione The liver hydrolyzes glutathione at a low rate

Shannon Have you made any calculations on what happens to the total dose administered? You have data on muscle, liver spleen and blood

Binkley I believe what you are asking is how well do we account for the glutathione we give

Shannon Yes

Binkley We account for 20 to 30 per cent

Shannon Twenty to thirty percent?

Binkley Yes

Shannon When you add in fragments derived from glutathione, how much further does that go up?

Binkley It brings it up perhaps to around 50 to 60 percent

Shannon What happens to the remainder?

Binkley We have not analyzed certain tissues

Shannon You have the bulk, the spleen, liver, kidney, muscle and blood

Binkley I think 60 percent

Shorr How rapid is the rate of glutathione hydrolysis?

Binkley Extremely rapid

Shorr Is it more rapid under anaerobic conditions?

Shorr Yes

Shannon It is surprising you can account for so much of such a labile substance in a fairly long interval

Binkley We are determining the fragments Cystine although degraded is not degraded too fast by tissues other than liver

Thorn Did you get any appreciable fall in blood sugar levels when you administered your glutathione?

Binkley Actually there is an increase of blood sugar levels

Thorn What about man? Isn t it true that most of the nausea from the amino acid preparation is related to glutamic acid? Can you give very much of your combination to man without producing serious symptoms?

Binkley We have not given any of the materials to the human as yet There is the problem of the pyrogens

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BIOASSAY OF SODIUM RETAINING CORTICOIDS AND SOME CHANGES IN EXCRETION OF THESE SUBSTANCES IN DISEASE*

JOHN A LUETSCHER JR and QUENTIN B DEMING

Department of Medicine Stanford University School of Medicine

WHEN DR PITTS asked me to present this material I emphasized to him that our work is in a preliminary stage and that many fundamental questions remain to be answered

This work started with some observations on nephrosis in collaboration with Dr A D Hall(1,2) Dr Fremont Smith expressed earlier his dissatisfaction with the time honored concepts of the sequence of events in hypoproteinemia as being due to loss of protein leading directly to hypoproteinemia and edema In studies of treatment of nephrosis with albumin we found that replacement of the circulating albumin of patients with nephrosis was regularly followed by modest increases in albumin concentration and in colloid osmotic pressure but little change in total protein concentration A large increase in plasma volume ensued accompanied by increased clearances of inulin and creatinine accelerated water output and rise in serum sodium concentration In about half the patients the whole thing stopped there and no effective elimination of edema was achieved In the other half of the patients the urine sodium increased and an effective diuresis followed The total serum protein concentration rose as diuresis progressed These observations suggested that urine sodium excretion was the limiting factor in the diuresis after the initial stage of water diuresis had been passed and that the serum protein concentration could be raised only after diuresis of sodium had been established

We then attempted an analysis of this data on the basis of possible causes of excretion of sodium to fail under these circumstances and came to the conclusions that a large number of factors were involved and that our experiments were not adequate to define any single factor responsible for sodium elimination or its failure Albumin deficiency a low plasma volume a low serum sodium concen

* This investigation was supported by a research grant from the National Heart Institute

tration, and a low glomerular filtration rate were frequently associated with sodium retention, but the return of any one of these factors to normal was not necessarily followed by increased sodium excretion Furthermore, when the sodium excretion was increased, it could not necessarily be correlated in time with any one of these factors It appeared that a low glomerular filtration rate could impair sodium excretion or even, at extremely low levels of filtration rate, could prevent sodium excretion But at higher rates of glomerular filtration or, we should say, inulin clearance, the tubule could still restrict the output of sodium to very low levels You are familiar with the nephrotics with supernormal filtration rates, which are another reflection of the same phenomenon(3)

In casting about for a reason for the impairment of sodium excretion in these edematous patients, it seemed worth investigating further the possibility that something outside of the kidney might be playing a role It seemed to us that the deficiency in albumin in the nephrotic might lead to a low plasma volume which in turn might stimulate both posterior pituitary-like activity, as one might see it after hemorrhage or fainting, and adrenal activity like that due to any comparable stress Some information has been published during the course of our studies which indicates that there are increased levels of total corticoids in the urine in heart failure and in certain patients with toxemias of pregnancy with edema(4 5) These data did not seem conclusive, since we know that reducing and glycogenic corticoids vary greatly in their sodium retaining effect So we started out in the simplest way to measure the sodium retaining activity of the urinary corticoid fraction We have also attempted to measure antidiuretic activity in urine and in serum Dr Quentin B Deming has carried the bulk of the work in these [illegible] procedures I would like to say that he has really done a most [illegible]

Our antidiuretic hormone assays have been limited by problems connected with the available methods Assays of urine of feeble activity by intraperitoneal injection in rats have given us very questionable results The concentration of urine before injection leads to all sorts of errors Dr Deming has not been able to elute the material quantitatively from any chromatographic adsorbent so far

Assays of serum according to Dr Gaunt's method have given us occasional positive results of some interest, which I may mention

later in connection with one case We have done no really systematic studies of antidiuretic hormone excretion or the serum levels

The sodium retaining activity of the corticoid fraction is measured by bioassay in adrenalectomized rats(6) We started off in the measurement of this fraction following the method of Dorfman using radio-sodium(7) It soon became obvious that if one did not strictly control the rats intake of water and sodium there were large *spontaneous* variations in output So we put them on sodium free diets and gave them measured quantities of salt and of water by injection The sodium chloride was given as an isotonic solution intraperitoneally the night before the test and an injection of water was given intraperitoneally at the beginning of the test to stimulate urine flow Unfortunately using adrenalectomized animals with this water load we turned it into a sort of water test at the same time This has one merit in that it discriminates quite clearly the activity of some of the 11 oxysteroids which produce a diuresis and a loss of sodium in this test as compared with desoxycorticosterone for instance which causes sodium retention We gave up radioactive sodium in favor of the flame photometer for reasons of convenience

The solvents employed in the administration of these extracts to rats are of great importance because of the considerable activity of some of the conventional solvents In searching for a vehicle which did not affect the sodium excretion in the rats and still would be a good solvent for the urinary extracts we fell back on ethyl alcohol in very minute quantities This does not bother the rats as much as a larger injection of some other substances which you might think would be much less irritating It is an excellent solvent and does not affect the rats output of sodium

A twenty four hour specimen of urine is collected to avoid possible variations from day to night This is acidified with hydrochloric acid to pH 1 5 and extracted immediately with chloroform The days urine extract in chloroform is dried evaporated and taken up in a minute quantity of ethyl alcohol A twenty minute sample is then injected into each adrenalectomized rat We have expressed all of our doses in terms of the time during which the patient excreted the urine equivalent to the dosage rather than in terms of volume of urine in order to avoid the influence of urine flow on the assay The rat is then placed on a metabolism cage and urine is collected for the next five hours measured and analyzed for sodium content

tration and a low glomerular filtration rate were frequently associated with sodium retention but the return of any one of these factors to normal was not necessarily followed by increased sodium excretion Furthermore when the sodium excretion was increased it could not necessarily be correlated in time with any one of these factors It appeared that a low glomerular filtration rate could impair sodium excretion or even at extremely low levels of filtration rate could prevent sodium excretion But at higher rates of glomerular filtration or we should say inulin clearance the tubule could still restrict the output of sodium to very low levels You are familiar with the nephrotics with supernormal filtration rates which are another reflection of the same phenomenon(3)

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Our antidiuretic hormone assays have been limited by problems connected with the available methods Assays of urine of feeble activity by intraperitoneal injection in rats have given us very questionable results The concentration of urine before injection leads to all sorts of errors Dr Deming has not been able to elute the material quantitatively from any chromatographic adsorbent so far

Assays of serum according to Dr Gaunt s method have given us occasional positive results of some interest which I may mention

between 20 and 40 minutes of urinary extract from a patient from normals as well as the more active extracts from the cardiac patients and the nephrotics Below this level the response of the rats decreased in the expected way When more than this dose of extract was given no further increase in sodium retention was caused by the higher dose even though the response was well below the maximum effect that could be elicited from those animals by larger doses of desoxycorticosterone For this reason we can speak only of the activity of these crude extracts under the specific conditions of the experiment and cannot speak in more general terms I think this data emphasizes the need for fractionation and for further study of interfering substances or whatever is the limiting factor in the response of the rats to these extracts

We studied the effects of a small series of crystalline steroids Desoxycorticosterone or its acetate was used as a standard The rats were sensitive down to approximately one microgram of DOC or DCA with a fairly straight line response from there up to five micrograms Above that dose the response to further increases in dose falls off until a plateau is reached at a dose of about 10 to 25γ Cortisone at 10 micrograms gives no response At 100 micrograms cortisone produces a striking diuresis of water in these adrenalectomized animals and more sodium appears in the urine We have not run any other adrenal steroids and have not run competitive studies between desoxycorticosterone and cortisone Testosterone estradiol and progesterone are weak sodium retainers active only at high dosage(6)

In a series of individual and pooled specimens of human urine we determined the activity of twenty minutes equivalent of urine from normal individuals and found a mean activity equivalent to 1 5 micrograms of desoxycorticosterone per twenty minutes of extract Eleven out of twelve individual assays have shown levels of 2 3 micrograms or less for twenty minutes There has been no difference in activity of urine from men and women as far as we can make out in this small sample The effect of the menstrual cycle on this assay is apparently small although this has not been systematically tested

In one group of patients tested by the original method in which we simply estimated the factor by which the sodium excretion was reduced the crude assays indicated that there was relatively little activity in the extracts from normal patients and control patients

The assay takes three days to perform Each rat serves as its own control On the "control" day it receives no injection or a control injection of alcohol On the test day it receives the extract to be tested On the *standard* day it receives five micrograms of desoxycorticosterone acetate In order to assign a quantitative measure of activity we have used two measures The first one is the factor the ratio between the output of sodium by the rat on the control day and the output on the test day In other words an active extract might reduce the excretion of sodium by a "factor" of two compared with the control day Groups of nine rats are used for each assay and the logarithms of the individual factors are averaged to obtain an average factor The log of the factor obtained is approximately proportional to dosages of DCA between 1 and 5 micrograms for each rat group If a line is drawn on semi log paper from the origin (factor 1 dosage 0) to the point representing the response to the standard injection (factor determined dosage 5γ) the activity of the unknown can be assigned an equivalent in micrograms of DCA

When we started to test this assay procedure we encountered another disturbing variable If one uses rats immediately after adrenalectomy there are changes from day to day If one waits too long one has a number of sick or dying rats We have worked in the intermediate stage of about three to five days after adrenalectomy In order to avoid the day to day variation we adopted the suggestion of Dr A G Spencer who was working on a very similar method last year at Columbia(8) We divided each group of nine rats into three subgroups which received the control standard and test injection respectively on each day The subgroups were rotated each day so that at the end of three days each subgroup had received the three different injections on different days

The reproducibility of these assays has been surprisingly good since the assay has been going smoothly If for any reason an assay is suspected of being erroneous it is repeated The second assay is within plus 10 or minus 10 percent of the duplicate assay if we are lucky and as badly off as 20 percent on an occasional bad day

With crude extracts of human urine one does not obtain a linear response as the dosage is increased Larger doses did not make the rats visibly sick nor did they impair the survival of these rats but it was obvious in plotting the dose of urine extract against its activity that a peak was reached when each individual rat received

Burnett These nephrotic patients all have edema?

Luetscher There has not been a strict correlation between the presence or absence of edema and any given level of activity of the urinary extract In general those patients showing the highest assays have been uniformly edematous and those patients with the lowest assays have been almost free of edema However I dont want to suggest in any way that this is the only possible cause of edema in nephrosis or any other condition All we are trying to do is to introduce another complexity into your thoughts on this subject

The series of untreated nephrotics now comprises twelve patients In these patients the mean assay is 3 8 micrograms with a range of 1 7 to 8 4 micrograms Eight of these twelve patients have been above 2 3 micrograms You will remember that eleven of twelve controls assayed 2 3 micrograms or less

Shortly after we became convinced that these levels were unusually high in the nephrotics cortisone became available If the activity which we were measuring arose in the adrenal cortisone might be expected to reduce the excretion of this abnormally active material Of course there are a great number of other possible explanations of the various effects of cortisone in nephrosis We wondered whether it might have some influence on the disease process itself We wondered whether we might see some antagonism of antidiuretic activity if the posterior pituitary was involved in the phenomenon We could speculate at length but first let us consider some data from patients with nephrosis under treatment with cortisone

In Figure 2* the assay levels are indicated in micrograms per twenty minutes as before Here is a five year-old girl who has a very moderate amount of edema She is only about a kilo above her ideal weight She has a modest proteinuria about a gram and a half a day She has a low serum protein level of 4 gm percent She is putting out a small urine volume containing modest amounts of sodium With the administration of 100 mg of cortisone a day for six days one sees an increase in the level of proteinuria to double the original low level a complete shutting off of urinary excretion of sodium and an increase in body weight which had been stationary before treatment After the discontinuance of treatment there is a release of water and sodium in the urine a sharp fall in the

* See p 22

from the hospital wards We encountered much more active extracts in patients with cardiac failure and in edematous patients with the nephrotic syndrome Some patients with hypoproteinemia hypercholesterolemia, and proteinuria, but without edema did not give a positive assay So there was a rough correlation with the presence or absence of edema(6)

When the assay was improved by the modifications described we began to express the results in micrograms of DCA We can now compare twelve patients with nephrosis with an equal number of normals and hospital controls (Figure 28) At a level of about 2.3 micrograms one can draw a fine artificial line which roughly separates our group of normals and patient controls from the edematous nephrotics with the high assays The most striking increases in activity have been observed in patients with the edema of nephrosis and in those with heart failure Levels above average normal have been noted in one case of acute pericarditis with effusion and signs of tamponade, one patient with chronic constrictive pericarditis and in two hypertensive patients uncomplicated by other factors One hypertensive in cardiac failure and a patient with malignant nephrosclerosis and mild cardiac failure have given a high assay

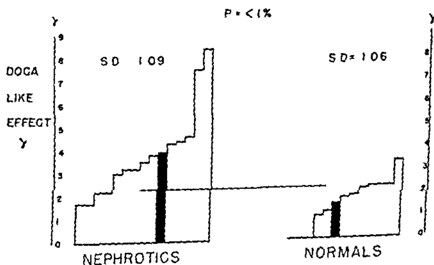


FIGURE 28

Comparison of sodium retaining activity of urinary corticoid fraction in nephrosis and in normals

the administration of 100 mg of cortisone a day we observed the diphasic response which has been referred to before First there was an increase in body weight and proteinuria Then after a week of treatment there was an increased urine volume a fall in body weight a diminution in proteinuria and small increases in serum protein and albumin concentrations A small increase in urine sodium also appeared At this time an assay indicated some reduction below the control level of activity in the urinary extract

Then following the withdrawal of cortisone there was a great increase in urinary volume and sodium The body weight fell 12 kilos below the control weight Proteinuria became less than 200 mg per day At this time there was a fall in the assay to a normal level Improvement seemed to occur regularly in these patients at about the time the eosinophils were rising after the end of cortisone administration We interpreted this coincidence in time as evidence

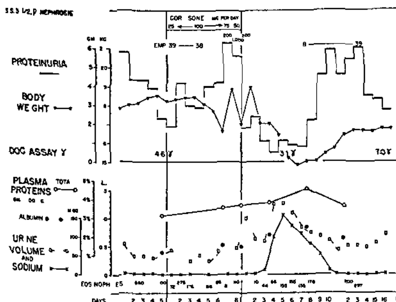


FIGURE 29*

Brief remission interrupted by infection

he

s
f

(In press)

proteinuria down to levels of 40 mg a day in this instance and an increase in the serum protein and albumin concentrations to levels approaching normal In this particular instance, we were unable to demonstrate any effect on the level of sodium retaining activity, which was within normal limits before treatment

Figure 3* shows some changes in a seven year old boy with the nephrotic syndrome, in this case with massive edema a proteinuria of 4 gm a day, a very small urine volume, essentially no sodium in the urine, and very low levels of total protein and albumin concentrations This patient's urine gave the highest assay we have observed in any patient We have been impressed with the very high levels of activity in many of the children with nephrosis and having no sound basis for the correction for body weight, we present these data as observed We were influenced, too, by the coincidence that at this time Dr Harold Faber asked us to see a baby three months old with Lawson Wilkins'(9) syndrome of congenital adrenal hyperplasia of the androgenic type with the lack of the sodium retaining hormone of the adrenal and excess of androgenic hormones I was astonished to discover that this infant required just as much desoxycorticosterone for maintenance as an adult In a casual search of the literature, this appeared to be the rule

Janeway That is a peculiar situation, isn't it Dr Thorn in which you don't have adrenal insufficiency in the ordinary sense but you might say you have adrenal dysfunction with disproportion in the production of different hormones, the adrenal putting out huge amounts of androgen We find they need two or three times the ordinary dose for maintenance I don't know that you can extrapolate from that to the needs of normal children

Thorn No

Dock This condition persists throughout life?

Janeway They are born with it

Thorn The 11 oxy methods give pretty close correlation of small children and adults on the excretion basis This does not of necessity of course refer to secretion of hormone

Luetscher In any case, here was a youngster with the severe form of nephrotic syndrome, with a very high control assay During

* See p 23

until eosinopenia was noted There was a very small output of urine, and the weight was steadily increasing Treatment was interrupted because of the rise of the serum potassium to 7 5 mEq per L After treatment, the weight gain proceeded as before There was no effect on proteinuria, and no release of sodium There was some increase in serum protein concentration The appetite improved greatly Increasing effusions caused respiratory embarrassment and Southey's tubes were used to draw off much of the edema The administration of concentrated human serum albumin was followed by diuresis and by a fall in the activity of the urinary corticoids

Figure 31 shows a similar response in a twenty year old man with the nephrotic syndrome Neither diuresis nor reduction in sodium retaining activity followed a brief course of cortisone but the level of the assay fell with the diuresis after the administration of albumin

Pitts Could you summarize there?

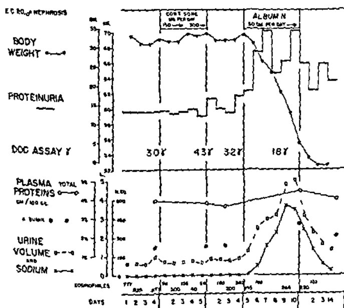


FIGURE 31

Failure of a brief course of cortisone to affect edema or level of sodium retaining corticoids with subsequent response to albumin

of a release from the effects of cortisone, not only on the eosinophils but on the kidneys and on the excretion of salt-active corticoids

Such a good clinical response has followed cortisone treatment in approximately half of the patients Even when it does occur, it may be very rapidly interrupted by an intercurrent infection Figure 29 shows the course of a three-year-old girl who developed a urinary tract infection after her diuresis There was an immediate rise in proteinuria and a disappearance of urinary sodium At this time the assay, which had decreased after the end of treatment, increased to a level higher than that in the control period

In those patients who failed to respond to cortisone, we have seen if anything a slightly higher sodium retaining activity in the urine after treatment than we saw during the control period Figure 30 shows the course of a three year old boy treated with cortisone We were uncertain as to the proper dose, and increased the dose

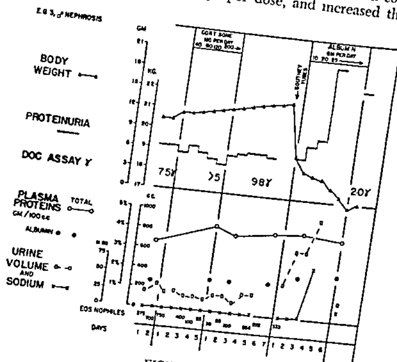


FIGURE 30

Failure of a brief course of cortisone to affect edema or level of sodium retaining corticoids with subsequent response to albumin

Note increased serum protein and albumin levels during cortisone administration

until eosinopenia was noted There was (((urine and the weight was steadily increasing T t rupted because of the rise of the serum pot (L After treatment the weight gain proce) was no effect on proteinuria and no releas (ll some increase in serum protein concentrat Γ proved greatly Increasing effusions caused t ment and Southey s tubes were used to draw il) t The administration of concentrated hum t (followed by diuresis and by a fall in the act t) corticoids

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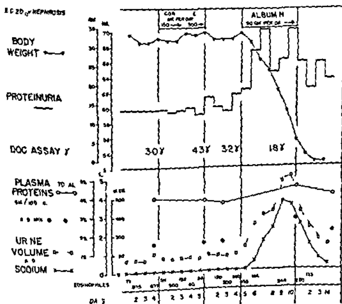


FIGURE 31

Fa lure of a br f course of cort sone to affect edema or le el of retaining cort o ds w th subsequent respon e to album n.

Luetscher Eight of fourteen patients have had a diuresis after cortisone treatment In three of the eight there is some doubt that cortisone was responsible for the improvement All except one of the eight had a reduction in proteinuria Two patients had a nearly complete remission in the sense of a fall of proteinuria to a few milligrams per day and a rise in serum protein concentration to essentially normal levels In the other six patients diuresis has failed to occur after the administration of cortisone We have not been able to determine why this failure occurred It is not related to the apparent etiology or activity of the renal lesion occurring both in patients with so called pure nephrosis and in those with evident glomerular nephritis We have not been able to divide the successes from the failures in terms of dosage or duration of treatment

To summarize the assays Of ten patients on whom assays have been done five patients have had a diuresis after treatment and three of these had had high assays before treatment Assays on these three patients fell after treatment The other two patients who had diureses gave assays in the normal range before treatment and these were unchanged after treatment Five patients who failed to show a diuresis after cortisone treatment all showed higher assay levels after the administration of cortisone than before Two of these cases showed a fall in assay after a subsequent diuresis with albumin

Two questions immediately arise in connection with these results The first one is What is the effect of a low sodium diet on the activity of the corticoid fraction? We have not yet set up systematic experiments to clarify this point Three patients on low sodium diets comparable to those in the nephrotics have shown normal levels of sodium retaining activity One of our nephrotics on a normal diet gave one of the highest assays that we have seen Sodium intake was not a major factor in these patients On the other hand we cannot rule it out in the other patients

The second question is whether diuresis *per se* might account for these results If one thinks of it in terms simply of a high water output we can say conclusively that this is not the case since we have seen situations in which the water output has increased while the assays remained high although sodium excretion remained quite low So simply a large urine volume is not the reason for the assay level to fall

There have been other important changes during the treatment of these patients in addition to the changes in the assay figures The creatinine clearance is usually increased during treatment with cortisone or ACTH This increase may begin when the urine sodium is very low The creatinine clearance is often falling when the peak of diuresis is reached after the end of treatment There has often been elimination of water during the latter days of treatment which has sometimes resulted in a considerable loss of weight and increased serum sodium concentration There have also been increased con centrations of serum albumin and in some cases of total protein

All of these changes are in the direction of normal but at the end of treatment it is very difficult to look at one of these patients and predict from the extent of these changes whether he is going to be one of the 50 percent who are going to have a diuresis or of the 50 percent who are not going to show a diuresis However when you look at the groups as a whole there is a higher average glomerular filtration rate and a higher average serum sodium at the end of treatment in those patients who are going to respond

I should like to mention briefly the effects of ACTH in the nephrotic So far five out of six patients have had a diuresis There is little question from these results and those reported by other students of the disease that ACTH is probably a more effective agent for clinical treatment However from an experimental stand point it leads to a much more complicated situation We started off with cortisone largely to try to keep our experiments as simple as possible We don t have enough data as yet from the bioassay to indicate with any certainty what the trend of the bioassays in patients treated with ACTH is going to show In attempting to explain the diuresis which occurs during ACTH treatment we have insufficient data to form a satisfactory basis for hypothesis

Figure 4* shows some changes in a sixteen year old girl with the nephrotic syndrome who was treated with cortisone with albumin and later with ACTH This is the only one of fourteen patients treated with cortisone and with albumin in whom no significant amount of edema was eliminated There was loss of edema during and after the subsequent administration of ACTH Here one can compare the effects which occurred during these different treatments We like to emphasize the diphasic changes in edema during cortisone and ACTH administration with the

* See p 24

corresponding changes in urine volume Urine flow is depressed during the early stages but usually increases again on later days of treatment In Figure 4* you can see this later release of water without change in sodium excretion with corresponding fall in body weight and rise in serum sodium concentration A similar increase in urine volume occurred during ACTH therapy at first without excretion of sodium Then as the serum sodium concentration approached normal significant quantities of sodium appeared in the urine Finally there was a more profuse diuresis after cessation of ACTH The changes in serum sodium are in the direction of a dilution and subsequent concentration of the serum sodium levels but this may not be the only factor involved

Attempts to correlate the changes in sodium excretion with glomerular filtration rate alone have met with little success In this patient as in most of our other patients there were considerable increases in creatinine clearance which have been checked from time to time with inulin indicating that a considerable increase in glomerular filtration rate has occurred during treatment with cortisone without any effect on the urine sodium There is also an increase in creatinine clearance during the administration of ACTH but the peak is slightly out of phase with the peak of sodium excretion The serum sodium level appears to exert an effect on the appearance of sodium in the urine but there is only a rough correspondence between the tubular load of sodium and the quantity of sodium in the urine There seems to be a number of factors involved and it is unlikely that any one factor will explain all the changes

In summary the assay data so far indicate that we have a very high control level in a number of edematous patients with heart failure or with the nephrotic syndrome We are not suggesting that this is the whole story of edema or a cause of disease It is simply a contributory factor which appears to play a role in many of these patients We wondered whether perhaps this might be a compensatory factor evoked by the deficiency of circulating protein in the nephrotic syndrome or by the reduced cardiac output in heart failure If increasing such activity is followed by increased proteinuria(10) in the nephrotic syndrome it is conceivable that a vicious circle might be set up in which the attempt to retain salt and water and to raise the circulating blood volume might

* See p 24

aggravate the proteinuria This is mere speculation but might explain the prolonged remissions which have followed therapy Much longer observation of patients will be necessary to determine possible effects on the underlying disease

We have emphasized the correlation of the activity of these urinary extracts with the edema of nephrosis without meaning in any way that this is the only factor involved in the production of edema It is obvious that protein deficiency a low plasma volume low serum sodium concentration and low glomerular filtration rate are all important

We would appreciate suggestions about where to go next We have much systematic work which we must do such as the effect of dietary sodium We feel that it is important to go on to some fractionation of these extracts Being interested in whether this was desoxycorticosterone or not we partitioned one of these active extracts between water and benzene We found that the abnormal activity just as Dr Pincus reported to your Conference on Adrenal Cortex last year on the activities of normal urine appeared in both the benzene and water fraction It does not go entirely into benzene as one might expect if it were desoxycorticosterone Dr Edward Hyman working with our group has done some preliminary chromatographic studies He has not found any material resembling desoxycorticosterone in his chromatogram of the active extracts When one of these active extracts is put on the column most of the components appear to be in the more polar fractions of the adrenal steroids Moreover we have failed to detect much activity in the extracts from urine of patients who are receiving DCA We would like to work on blood but have hesitated because of the very small quantities of circulating steroids We plan to do some studies on adrenal cortical extract and on more of the crystalline adrenal steroids to compare with the material which we recover from urine

If it turns out that the activity which we measure comes from the adrenals we may remember that there is still a lot of sodium retaining activity in whole adrenal extracts which apparently is not related to the crystalline compounds in the concentrations in which they are described in extracts One might rationalize the unusual activity by the concept of some autonomous r[illegible] on [illegible] the glomerular zone if you think that is where the hormones are produced What is the stimulus to this

sive activity or excessive secretion of the sodium retaining hormones? Under the circumstances I will say no more about these points but simply refer to the recent review of Sayers(11) on the regulation of adrenal activity Much more work is necessary before we can be sure that we are dealing with an adrenal secretion

DISCUSSION

Dock What is the level of excretion in Addison s disease?

Luetscher One case untreated with DCA fell in the low normal range The problem is that with the bioassay a very low activity is meaningless Let us say when we inject a microgram and a half of desoxycorticosterone into each of a group of rats that it may influence this group of rats to retain sodium equivalent to a factor of let us say 1 5 But we know that given a control injection containing no active material some rat groups may give a factor as high as 1 4 Therefore in the lowest levels of activity it is not possible to talk about the presence or absence of activity or to make an accurate quantitative assay

Ralli Dr Pincus has perfused isolated beef adrenals with 11 desoxycorticosterone Its perfusion produced material which when isolated in sufficient amount was characterized chemically as corticosterone or compound B in Kendall s list Pincus infers that this indicated the ability of the gland to oxygenate in the 11 position and to produce the 11 hydroxy group(12) This may or may not have some bearing on what you have been doing Another point is that the method of assay that you are using might not detect what was being excreted

Thorn Stahl in Strasbourg is actively at work on the metabolism of desoxycorticosterone in patients with liver disease Such patients handle DCA very differently from the normal It is also true that many arthritic patients reveal impaired liver function and some even exhibit amyloid disease It might be that one obtains much more complete metabolism of DCA under these circumstances than in a normal subject

It would also be desirable to check an Addisonian maintained on a constant dose of DCA and also an Addisonian on 100 or 200 mg of cortisone to be certain that there is no conversion to DCA An absence of DCA in patients with intact adrenals treated with cortisone could reflect an inhibition of endogenous DCA formation

Luetscher We have studied some patients who have been on high doses of cortisone for the treatment of arthritis Some of these patients have shown high normal levels but we have seen no extravagantly high levels

In answer to Dr Ralli's question I think Dr Pincus observed that the adrenal could convert 11 desoxycorticosterone to corticosterone That is presumably not the normal method of degradation of desoxycorticosterone in the body The fact that very little 11 oxysteroid is demonstrated after the administration of desoxycorticosterone indicates that in all probability very little goes in that direction

Thorn We have observed one patient with a mitral and tricuspid stenosis in marked edema with very low sodium level who on cortisone therapy developed normal sodium levels much like the nephrotic This was the first time in a long period of hospitalization the normal serum was obtained It is to be noted however that we administered potassium chloride to the patient with cortisone

Burnett Before or after?

Thorn The serum sodium returned to normal after cortisone therapy Did you ever notice chloride excretion twenty four hours before an increased sodium excretion of either ACTH or a dissociation of those two as a preliminary to sodium excretion?

Luetscher We have not measured chloride excretion That is on the agenda which has become much longer than the things we have been able to do

Ralli Is it possible that the diuresis that occurred during the administration of ACTH was due to substances in the pituitary extract other than ACTH?

Luetscher We assumed there were two phases the first one the diuresis of water without sodium We had thought of it as a possible anti antidiuretic action of the type described by Dr Gaunt In the sodium release during treatment we were interested to observe in one of our patients that the eosinophils rose simultaneously while in another one there was no convincing "escape" to judge by the eosinophils We are continuing to look for hints that the adrenal is somehow shifting gears at this point We plan to measure the 17 ketosteroids and some other conventional indices of adrenal activity during these changes to see whether we could show any

change in adrenal activity coinciding with the changes in urinary output

White Do I understand correctly that in these nephrotic cases during the period of administration of cortisone the proteinuria usually increases or does not change and then drops after you stop giving it? Is that the usual thing?

Luetscher Unfortunately, the usual picture is that nothing happens to the proteinuria after cortisone When the proteinuria changes, there is usually an increase during the first few days of cortisone administration, followed by a decrease in protein loss at the same time that one sees release of water and sodium If a real improvement is to occur, it occurs in the two or three days following the end of administration of cortisone

White How long does the protein output stay down?

Luetscher In some patients, the proteinuria reappears very quickly and returns to the pretreatment level, either after an infection or without obvious cause On the other hand we have two patients who have now gone between eight and nine months with no evidence of a return of proteinuria to the pretreatment level

White I was just wondering if there is any evidence of an increased production of cortisone in measles, more than in most other diseases

Luetscher I would like to say only one thing Our one failure with ACTH treatment confounded us by getting chicken pox one month later and having a magnificent diuresis

Fishberg In one patient I had a remarkable diuresis with measles and that patient relapsed I gave ACTH and again obtained a very fine result

Janeway Our group has been interested not specifically in the hormones, but in the problem of nephrosis for the past six or seven years I think the first point which I am sure Dr Luetscher will admit, is that anything that you do with a chronic disease like this has to be done against the background of what you know about its natural history If you get a nephrotic in a bed in a hospital and leave him alone, very often you will see regular fluctuations in body weight without any treatment A few times we have been able to get complete balance studies during such weight changes It looks as if fairly constantly you are having shifts of water into cells and

then out of the cells without any change in sodium excretion Apparently you can have changes in intracellular water going on which may account for these rather minor fluctuations

The second point is spontaneous diuresis which can occur at the most unexpected and embarrassing moments I was interested in looking at your Figure 4* Dr Luetscher where ACTH appeared to initiate a very remarkable diuresis Was that it? It had all the characteristics of the type of diuresis which may occur in these patients out of the blue There was a much sharper drop in proteinuria than we have usually observed with administration of ACTH I think it is not beyond the realm of possibility that such a diuresis might have happened whether the patient got ACTH or not Certainly these patients are in a condition where the slightest stimulus of almost any sort can set in motion so many mechanisms we dont understand that it is terribly difficult to interpret anything that is not repeatable again and again We have probably fallen into the trap of confusion but have restricted ourselves entirely to ACTH in these patients because we wanted to learn as much as we could about one agent and thus have not yet used cortisone at all We put all patients on 100 mg a day for ten days and they have a diuresis with I should say about 60 to 75 percent regularity just prior to or on withdrawal of ACTH One patient had a slight diuresis each time the drug was stopped as the eosinophils rose Following the third and most intensive course the eosinophils only returned to normal very gradually but this is when the weight came down the urine volume rose and the sodium excretion rose very sharply In that patient the changes in proteinuria were not very striking While there is some reduction the diuresis proceeded with continuing proteinuria It was only following the diuresis that proteinuria dropped off to much smaller amounts I should say the one difference we have seen in patients who received ACTH from those to whom we have given measles — and we have a series of about fifteen of those children now — is that following measles proteinuria diminishes very sharply just at the time or before the diuresis is initiated whereas in general in our patients who have been given ACTH the total output of protein per twenty four hours in the urine has really changed relatively little I dont think that is a terribly significant fact but it is about the only aspect of the response to these two stimuli that appears to be somewhat different We have treated twenty one patients now for a total of

* See p [illegible]

twenty nine courses and the response would appear to be dependent more than anything else on the duration of the disease

Dr Luetscher spoke about pure nephrosis and glomerular nephritis I won t get into that controversy except to say the more we get into studying the disease in children the more we are inclined to believe it is one disease that it is merely a matter of the intensity and duration of the process which gives you the different clinical picture This statement is based on work by Dr Metcoff and Dr Kelsey(13) who have made many observations of glomerular filtration rate by inulin and thiosulfate clearance both done in checking each other and agreeing fairly well at different stages of the disease in these patients The two striking findings have been first that glomerular filtration rate is always reduced in the patients that we have observed in the first few months of the disease and second that supernormal clearances may be observed in some of these patients from two to six months after onset Subsequently clearances in these patients may fall to low levels and this has been associated with the appearance of greater numbers of red cells in the urine whereas they are in a relatively normal range during the high clearance phase The main thing that I wanted to point out is the magnitude of the effect of ACTH on glomerular filtration rate in these nephrotic children In one patient it rose from 40 ml per minute up to 85 ml In another patient in whom clearance had fallen from the supernormal phase to quite low levels in ten days it has come back to nearly normal and clinically at least that patient is now doing extremely well What the explanation for these changes in glomerular filtration is is certainly far from clear These are a good deal more striking than the changes which Dr Burnett has seen in his normals with doses that are somewhat greater than the ones we have given Another thing which to me is very striking is that in several patients who have failed to respond with any diuresis to the administration of ACTH there has been a very striking change in the ability of the kidney to carry out its biochemical functions Just the other day I saw a patient whose NPN had risen from 45 over a three months period to a level of 98 who was becoming acidotic and for whom we gave a very bad prognosis ACTH was given without any effect on water balance whatsoever but ever since that time his NPN has been normal his acidosis has been repaired and his tubules are apparently able to carry out the regulatory job which they could not do before

Burnett Did the hematocrit fall?

Janeway ACTH tends to increase the red cell mass but the plasma volumes remain almost exactly the same

White The behavior of the filtration rate in dogs on ACTH is like that seen by Dr Burnett in the normal humans Neither normal nor hypophysectomized dogs have shown any increased filtration rate on ACTH We gave much smaller doses than those mentioned here One out of three of our hypophysectomized dogs did show a 30 percent increase in filtration with ACTH but so did one out of two adrenalectomized dogs show the same increase on ACTH

Thorn We have been interested in this mechanism and you might be interested in some of our observations When we first gave ACTH we gave it for a short period i e five to seven days The diuresis we observed occurred following the withdrawal of ACTH and we felt that in part at least the inactive adrenal following withdrawal of hormone was responsible for the increased sodium chloride and water loss However in later experiments with prolonged ACTH administration a diuresis was observed on ACTH in the presence of a very active adrenal secretion

Another interesting point is that during the period of diuresis if the patient is tested with pitressin or with desoxycorticosterone the kidney tubule appears to be relatively refractory to these agents just the reverse of adrenal insufficiency In other words a standard dose of desoxycorticosterone 20 mg or a standard dose of pitressin did not give the sodium or water reduction which we usually see in patients with adrenal insufficiency and we wondered from that whether there was not as a result of the ACTH treatment an incapacity on the part of the kidney itself to react normally to these two agents

Having studied a number of patients with acute nephritis subacute nephritis and nephrotic syndrome I have come to the conclusion that ACTH and cortisone have little beneficial effect on kidney disease and I think that this may also be true in liver disease It strikes me that this is very interesting when one considers the beneficial effects of these hormones in such a wide variety of nonspecific inflammatory diseases With nephrotic patients the results are different Here the diuretic actions of ACTH and cortisone are very satisfactory I do not believe the course of the disease is really modified specifically however We recognize that in many patients any agent which will induce the disappearance of edema

is likely to be followed by a decrease in protein excretion and a more or less prolonged remission

Burnett Our experience with cortisone in nephritis is that it may actually make the disease process worse Although renal function was improved by cortisone proteinuria and hematuria were also markedly increased during the administration of this steroid(14)

Lauson I would like to remark as long as we have come back to the nephrotic syndrome again that it seems possible to achieve a certain amount of unification of concept from the various studies on ACTH albumin therapy and nitrogen mustard I have been impressed by the fact that when both nitrogen mustard and ACTH therapy result in diuresis there is a concomitant reduction in proteinuria an expansion of plasma volume and an improvement of the circulatory status — in other words a more adequate circulation As far as I know there has been a rise in glomerular filtration rate each time that there has been a successful diuresis Albumin does the same thing except that diuresis occurs after direct expansion of blood volume and rise of filtration rate without a decrease in proteinuria On the contrary the bulk of the administered albumin goes out in the urine I think it is possible at present to look upon the nephrotic syndrome as a consequence of a single initial event namely proteinuria resulting in a condition of circulatory inadequacy Reversal of that circulatory inadequacy may well be the basis of spontaneous diureses or those induced by ACTH nitrogen mustard or albumin

I had a question I would like to ask Dr Luetscher on the technical aspects of making a chloroform extract of highly proteinaceous urine I wonder whether you have done the control experiments of adding to normal urine both plasma proteins and an adrenal steroid like pure desoxycorticosterone for example and then put that through the extraction procedure and assay to see whether in the presence of all the protein you can get out that little bit of steroid? Is there perhaps a possibility that you are getting something nonspecific out of this protein mixture that may have a salt retaining effect in the assay rat?

Luetscher This possible source of error is ruled out I believe by the low activity of the urines of those patients who have received concentrated human serum albumin If diuresis occurs in these patients the sodium retaining activity returns to normal These

relatively inactive extracts come from urines which contain more protein than the control urines which were highly active

Pitts You spoke Dr Luetscher about certain difficulties in finding in your solvents themselves some sodium retaining activity and your eventual choice of ethyl alcohol as being the least disturbing Could you amplify that a little more?

Luetscher We started out with the conventional method of suspending the extract in bland oil and encountered two problems In the first place many of the more polar steroids are quite insoluble in oil and it is very difficult to get uniform suspensions of urinary extracts We had to work with unstable suspensions in oil which seemed very unsatisfactory

Furthermore if you are taking up chloroform extracts into oil it is easy to contaminate the oil with traces of chloroform which may produce renal injury in the rats So we tried a number of vehicles which we thought would be better solvents and would be easier to work with in every way We ran into a lot of unexpected activity in control experiments with injections of the solvents(6) Of the solvents that were tried only polyethylene glycol and alcohol appeared to be both nontoxic to the rats and to have a lack of effect on urinary excretion of sodium We tested carbowax propylene glycol glycerine and carbitol When injected in the fairly large quantities necessary to dissolve the extracts the adrenalectomized rats showed either some disturbance of renal function suppression of water output or suppression of water and sodium output The only two that we found that were decent solvents for the range of compounds in which we were interested and which had no effect on the excretion of sodium or of water by the adrenalectomized rat were minute quantities of ethyl alcohol and polyethylene glycol

Gaunt Propylene glycol can be a toxic substance in the adrenalectomized rat

Luetscher There are a lot of interesting points that we tend to overlook. Certain bland solvents have been observed to increase the liver glycogen in the adrenalectomized rat There was one moment when we thought we could work out a fairly complete treatment for adrenal insufficiency from a mixture of ordinary solvents but unfortunately the pathological studies of some of these rats afterwards indicated that the large doses which we used were quite toxic

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